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# JOURNAL OF DAIRY SCIENCE

VOLUME XXXIII

JANUARY, 1950

NUMBER 1

## QUATERNARY AMMONIUM COMPOUNDS AS STERILIZING AGENTS FOR BACTERIAL SPORES

HAROLD R. CURRAN AND FRED R. EVANS

*Bureau of Dairy Industry, Agricultural Research Administration,  
U. S. Department of Agriculture, Washington 25, D. C.*

Since their commercial introduction in this country in 1937, quaternary ammonium compounds have been studied intensively with special reference to their antiseptic and bactericidal properties. The sporocidal activity of these compounds has received much less attention and the published reports reveal a wide divergence in essential findings and conclusions.

The reports of Kayser (14), Dunn (5), Zeissler and Gunther (25), Schubert (24), Seales and Kemp (23), Neufeld and Schütz (20), Hausam *et al.* (8), Du-bois and Dibblee (3) and Mueller *et al.* (19) indicate that quaternary ammonium compounds are not particularly effective against bacterial spores. Complete killing of spores in the absence and presence of organic matter was achieved only when the reagents were used in relatively strong concentration (1 to 10 per cent) and the exposures were maintained for considerable periods, frequently at elevated temperatures.

Other investigators using similar source material reported rapid (less than 15 min.) and complete killing of spores in water and broth substrates by low concentrations of quaternaries: 0.5 per cent at room temperature (11); 0.5 per cent at room temperature (9); 0.05 per cent, 37° C. (10); 0.2 per cent, room temperature (16, 17); and 0.250 to 0.0022 per cent, depending on the quaternary and organism used, unstated temperature (12). Green and Birkeland (7) stated that cetyl pyridinium chloride is an effective germicide for bacterial spores. Johns (13) reported that Roccal and Hyamine 1622 in 0.1 per cent concentration at 20° C. killed 99.9 per cent of the spores of *B. panis* in 1 to 3 sec. For the quick sterilization of heavily contaminated instruments, Brekenfeld (2) recommended the use of Zephriol (0.75 to 2 per cent) at boiling temperatures; the efficacy of this procedure was disputed by Zeissler and Gunther (25), a view also supported by the data of Schubert (24).

From this brief review, it is apparent that the efficiency of quaternary ammonium compounds as spore-killing agents is not clearly defined. The present study is an attempt to clarify this subject and to furnish a more satisfactory basis for the evaluation of these compounds as sterilizing agents. The term sterilizing agent is used here in its true sense, *i.e.* complete destruction of the test organisms.

Received for publication July 26, 1949.

In the reports cited, it is noteworthy that all except Hoogerheide (10) and Hucker *et al.* (12) dealt individually with a single quaternary ammonium compound, usually Roccal (Zephiran, Zephirol) and, for the most part, with a single organism. In the present study a representative group of quaternaries was tested against a variety of sporing species. In the study of sporocidal as distinguished from sporostatic activity, emphasis was placed upon those organisms not easily controlled by heat-sterilization methods.

#### MATERIALS AND METHODS

The following compounds were studied: Roccal<sup>1</sup> (alkyl dimethyl benzyl ammonium chloride), Onyxide<sup>2</sup> (alkenyl dimethyl ethyl ammonium bromide), Ceepryn (cetyl pyridinium chloride), Nopeocide K (dodecylacetamide dimethyl benzyl ammonium chloride), Hyamine 10X (diisobutyl cresoxethoxy ethyl dimethyl benzyl ammonium chloride), Hyamine 1622 (diisobutyl phenoxyethoxy ethyl dimethyl benzyl ammonium chloride), Emcol 888 (alkyl aryl pyridinium chloride), Tetrosan (alkyl dimethyl 3,4-dichloro benzyl ammonium chloride) and Amosol-1 and Amsol-2<sup>3</sup> (chemical components not revealed by manufacturer). These compounds were received in solid (crystalline) form or in concentrated aqueous solutions. The manufacturer's statement of concentration of active ingredient was accepted in calculations of quaternary concentration. The Amosols were mixtures of a quaternary with alkaline detergents; these two compounds were used in the proportions (by weight) indicated without adjustment of quaternary concentration.

The 10 per cent concentrations of the quaternaries were aqueous dilutions of the more concentrated products; lower concentrations were made by dilution of the 10 per cent solutions with suitable proportions of buffers<sup>4</sup> to yield with the inoculum 4 ml. of a final suspension of the desired pH and quaternary concentration.

The tests were made as follows: To a constant volume of buffer or aqueous solutions in tubes was added 0.5 ml. of the spore suspension and the contents mixed by moderate rotation of the tube; the quaternary then was added without touching the walls of the tube with the pipette and the tubes gently rotated and quickly immersed in a glycerine bath thermostatically controlled ( $\pm 0.5^\circ$  C.) at the desired temperature. The samples were agitated gently during the first 5 min. and at the end of the exposure period, after which 0.1 ml. quantities of the test suspension were pipetted into flasks containing 40 ml. of the subculture (detoxification) medium. The flasks were shaken gently and stored at the optimum temperature for growth of the organism. Evidence of growth and probable purity was verified in each case by microscopic examination of film preparations.

*Detoxification.* Several substances of potential value for the detoxification

<sup>1</sup> Also known as Roclina, Rodalon, Zephiran, Zephirol and Benzalkonium chloride.

<sup>2</sup> Also known as Quartol.

<sup>3</sup> A more recently developed product than Amosol-1.

<sup>4</sup> M/15  $\text{KH}_2\text{PO}_4$ , M/15  $\text{NaHPO}_4 \cdot 2\text{H}_2\text{O}$ , M/10 NaOH (stronger solutions of this compound were used when necessary).

of quaternaries were studied; these included agar, starch, lecithin in Tween, crystalline albumin and oxgall. A modification of the Lethen medium (lecithin in Tween 80) of Quisno and Foter (21) was adopted eventually; its composition was: Beef extract (Difco) 3.0 g., Peptone (Difco) 5.0 g., lecithin (soya)<sup>5</sup> 5.6 g., Tween 80<sup>6</sup> 40 g. and distilled water 1,000 ml.

Dubos (4) found that commercial Tweens contain sufficient unesterified fatty acid to inhibit the growth of small inocula of tubercle bacilli which, if removed or inactivated, eliminated the bacteriostatic effect. Since commercial Tween 80 in the required concentration was found to inhibit some of the sporogenic organisms, the purified<sup>7</sup> product was employed. This was without inhibitory activity for minimal inocula. The subculture (Lethen) medium was prepared as needed to minimize the slow hydrolysis which Tween undergoes in aqueous substrates.

The organisms used and their sources were: *Bacillus mycoides* 6462, *B. cereus* 401, *B. sphaericus* 4525, *B. circulans* 7049, *B. polymyxa* 8523, *B. brevis* 8185, *B. pumilus* 7061, *B. atterrinnus* 230 (all from N. R. Smith); *B. subtilis* 6, 15 u and *B. stearothermophilus* C<sub>2</sub>P<sub>3</sub> (American Can Company); *B. subtilis* LB (L. A. Burkey), *B. metiens* (W. A. Randall); *B. stearothermophilus* 1518, 26 and a mesophilic proteolytic anaerobe 3679 (National Canners Association).

The spores were produced on the surface of plain nutrient agar slopes contained in large flat-sided prescription bottles (those of 3679 in thioglycollate supplement broth). Time of incubation was 3 to 4 wk. at the optimum temperature of the organism. The spore crops were collected in the usual manner, filtered through cotton, washed several times and stored in distilled water at 3° C. The inocula were prepared by dilution of the stock suspensions. The concentration of viable spores during the exposure to the quaternaries, except when otherwise noted, was approximately 5 million per ml., that of the thermophilic flat sour types somewhat less.

## RESULTS

The quaternary ammonium compounds first were studied in different concentrations at 30° C. and pH 9.6 with exposure periods up to 30 min. Under these conditions no one of the compounds in concentrations up to 5 per cent consistently killed all the spores in the test suspensions within 30 min. When the temperature of exposure was 60° C., the same result was obtained. At exposure temperatures of 80° C., complete killing of the spores was first observed. The data (table 1) show that sterilization of the samples apparently was achieved by several of the quaternaries, but against only a limited number of cultures. The flat sour thermophilic species (1518 and 26), which were the most susceptible, did not grow in subculture after either 20 or 30 min. contact with three of the quaternaries. Doubling the concentration of the quaternaries at 80° C. and with no adjustment of pH (table 2) slightly increased the over-all sterilizing effectiveness of the compounds but reduced the sporocidal activity in specific instances.

<sup>5</sup> American Lecithin Co., Inc.

<sup>6</sup> Tween 80, Atlas Powder Co.

<sup>7</sup> Approved by R. J. Dubos for use in bacteriological culture medium.

TABLE 1  
*Sporocidal activity of quaternary ammonium compounds (1-20) at 80° C.*  
*(spores exposed in buffer solutions pH 9.6)*

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with:									
		Roccal	Onyxide	Ceepryn	Nopeocide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan	Amosol #1	Amosol #2
	(min.)										
<i>B. subtilis</i> LB	20	3	3	3	3	5	4	11	3	3	3
	30	5	3	3	3	5	4	30	3	4	3
<i>B. subtilis</i> 6	20	1	1	1	1	1	1	1	1	1	1
	30	1	1	1	1	1	1	1	1	3	3
<i>B. atterimus</i> 230	20	1	1	1	1	1	1	1	1	0	2
	30	2	1	1	1	1	1	2	1	0	0
<i>B. stearohermophilus</i> 1518	20	0 <sup>a</sup>	2	7	1	2	2	0	2	1	1
	30	0	0	2	1	2	2	0	7	2	2
<i>B. stearohermophilus</i> 26	20	0	0	0	1	4	4	0	4	1	1
	30	0	0	0	1	4	4	0	4	1	1
<i>Clostridium</i> sp. 3679	20	3	3	4	3	3	3	3	3	12	0
	30	4	3	3	4	3	3	4	3	0	3

<sup>a</sup> 0 = no growth.

TABLE 2  
*Sporocidal activity of quaternary ammonium compounds (1-10) at 30° C., no pH adjustment<sup>a</sup>*  
*(spores exposed in aqueous solution)*

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with:									
		Roccal	Onyxide	Ceepryn	Nopocide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan	Amosol 1#	Amosol 2#
(min.)											
<i>B. subtilis</i> LB	20										
	30										
	20	8	3	3	3	4	3	7	3	3	10
<i>B. subtilis</i> 6	30	8	3	3	3	11	3	5	3	5	4
	20	1	1	2	1	1	1	1	1	2	2
	30	1	1	2	1	1	1	1	1	2	4
<i>B. subtilis</i> 15a	20	4	2	2	2	5	3	14	3	2	2
	30	5	2	2	2	3	3	11	3	4	0
	20	5	9	3	1	2	2	0	3	2	2
<i>B. stearothermophilis</i> 1518	30	0 <sup>b</sup>	12	3	1	2	3	0	5	2	2
	20										
	30	0	0	0	3	14	1	0	0	3	3
<i>B. stearothermophilis</i> 26	20	0	0	0	3	12	10	0	0	3	3
	20	5	5	5	5	5	5	5	5	0	0
	30	5	5	5	5	5	5	5	5	0	0
<i>Clostridium</i> sp. 3679	20										
	30	5	5	5	5	5	5	5	5	0	0

<sup>a</sup> Amosols approximately pH 12, others ranged between pH 3.6-7.5.

<sup>b</sup> 0 = no growth.

TABLE 3

*Sporocidal activity of quaternary ammonium compounds (1-20) at 95° C. with variations in pH*  
(spores exposed in buffer solutions)

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with:											
		Roccal			Onyxide			Ceepryn			Nopocide K		
		pH			pH			pH			pH		
		6.4	8.0	9.6	9.6	8.0	6.4	9.6	8.0	6.4	9.6	8.0	9.6
		M <sup>a</sup>			M			M			M		
	(min.)												
<i>B. subtilis</i> LB	20	14	0	0	0	2	1	1	2	2	5	2	6
	30	35	0	0	0	2	1	1	8	2	4	5	5
<i>B. subtilis</i> 6	20	3	2	0	0	3	1	1	2	10	1	4	1
	30	3	3	0	0	3	1	3	2	3	1	4	1
<i>B. subtilis</i> 15u	20	7	0	0	0	2	2	3	4	2	2	3	1
	30	9	0	0	0	3	7	3	3	2	0	0	5
<i>B. atterimus</i> 230	20	0 <sup>b</sup>	1	0	0	1	1	3	0	2	0	0	7
	30	0	0	0	0	0	0	0	0	1	0	0	4
<i>B. steatothermophilus</i> 1518	20	2	3	0	0	5	3	4	0	0	1	3	3
	30	0	0	0	0	6	3	0	4	1	0	3	4
<i>B. steatothermophilus</i> 26	20	0	0	0	2	0	0	0	0	1	3	0	3
	30	0	0	0	0	0	0	0	0	1	4	0	0
<i>B. steatothermophilus</i> C <sub>1</sub> P <sub>1</sub>	20	10	0	0	10	8	0	0	0	1	2	0	4
	30	0	0	0	0	0	0	0	0	1	4	0	7
<i>Clostridium</i> sp. 3679	20	3	0	0	0	3	3	3	3	3	0	0	6
	30	0	0	0	0	3	0	3	3	0	0	0	3
<i>B. metiens</i>	20				1					1			3
	30				0					1			0

TABLE 3—(Continued)

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with:											
		Hyamine 1622			Emcol 888			Tetrasan			Amosol #1		
		pH			pH			pH			pH unadjusted		
		6.4	8.0	9.6	6.4	8.0	9.6	6.4	8.0	9.6	M	M	M
	(min.)			M			M			M			
<i>B. subtilis</i> LB	20	3	3	4	3	10	0	2	4	0	0	15	0
	30	3	5	5	12	14	0	0	4	13	0	0	0
<i>B. subtilis</i> 6	20	3	1	1	1	3	2	4	26	1	0	0	0
	30	3	1	1	2	3	8	4	2	0	0	0	2
<i>B. subtilis</i> 15u	20	2	7	5	4	0	0	2	5	15	0	0	0
	30	3	7	4	3	0	0	2	5	14	0	0	0
<i>B. atterratus</i> 230	20	3	2	2	3	0	4	0	1	0	6	0	3
	30	1	0	0	0	0	0	0	0	0	0	0	0
<i>B. stearothermophilus</i> 1518	20	1	3	3	3	0	0	4	0	0	3	0	0
	30	1	3	3	3	0	0	0	0	0	0	0	0
<i>B. stearothermophilus</i> 26	20	2	4	3	0	0	0	7	4	0	0	0	3
	30	4	4	3	0	0	0	0	4	0	0	0	0
<i>B. stearothermophilus</i> C <sub>2</sub> P <sub>2</sub>	20	2	4	2	0	0	0	10	0	0	0	0	0
	30	2	6	2	0	0	0	0	6	0	0	0	0
<i>Clostridium</i> sp. 3679	20	3	0	3	3	0	0	3	0	0	14	2	14
	30	0	0	0	3	0	0	0	0	0	0	0	0
<i>B. metiens</i>	20				0			1			0		4
	30				0			1					11

<sup>a</sup> M = buffer contained skim milk (1-500).<sup>b</sup> 0 = no growth.



TABLE 4  
*The activity of quaternary ammonium compounds (1-20) at 95° C. against spores dried in milk films on stainless steel strips (spores exposed in buffer solutions pH 9.6)*

Culture	Day on which growth in subculture was first observed after treatment with:										Amosol #1	Amosol #2
	Check	Roccal	Onyxide	Ceepryn	Nopcoide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan			
<i>B. subtilis</i> LB	1	7	4	4	4	5	7	10	8		1	1
<i>B. subtilis</i> 6	2	0 <sup>a</sup>	2	3	0	2	4	0	0		4	4
<i>B. subtilis</i> 15u	1	8	2	4	3	4	0	0	4		4	1
<i>B. stearothermophilus</i> 1518	2	0	0	3	3	0	0	0	0		1	0
<i>Clostridium</i> sp. 3679	2	0	5	3	0	0	0	0	0		0	0

<sup>a</sup> 0 = no growth.

Tables 3 and 4 show the results obtained when the spores were exposed at 95° C. in buffer substrates of varying pH, with and without traces of skim milk. Even at this near-boiling temperature, most of the compounds in 1-20 (5 per cent) concentration did not sterilize the suspensions. Roccal, the most generally effective sporocidal agent under these conditions, apparently killed all the spores of all the test species in 30 min. at pH 9.6 but not at the less alkaline levels. The diminished sporocidal activity at pH 6.4 and 8.0 was evident, also, with the other compounds. The influence of pH on sporocidal activity was revealed further by the observation that Roccal even in 1-10 concentration at 95° C. did not sterilize the *B. subtilis* 6 culture in 30 min. when distilled water at pH 7.5 was the substrate (unpublished data), although at pH 9.6 and 95° C. Roccal sterilized the suspension within 30 min. at 1-20 concentration. The pH of the Amosols was not adjusted, since the high buffering capacity made this impracticable; these formulations ranged from pH 12 to 13.

Traces of skim milk in the exposure substrate extended the time of sterilization in some cultures, this being most frequent with the thermophilic species. Emcol 888, Tetrasan and the Amosols sterilized the thermophilic and anaerobic cultures but were less effective against the *subtilis* types. The spores of *B. metiens*, unlike those of the other test organisms, are relatively sensitive to heat; in consequence most of the spores are killed by the higher heat treatment *per se*. Survival and growth of these organisms in subculture indicates a high degree of resistance to specific quaternaries on the part of very few relatively heat-resistant spores. Spores that survived contact with the quaternaries usually developed more slowly than those not so treated; however, most samples which finally became positive did so within 10 days. In exceptional instances, evidence of growth by survivors first appeared after 40 or more days of incubation.

The test spores developed readily in the basal (Lethen) medium in minimal numbers (10-12 spores per ml.). Accepted as evidence that the Lethen medium provided satisfactory inhibition of quaternary activity was growth in the Lethen broth by minimal number of spores in quaternary concentration equal to the highest used in the test, with momentary exposure of the spores to the quaternary before their transfer to the inhibitor. Results were recorded as negative when seven to ten samples, all treated alike, failed in each instance to show growth within 60 days; when, during this period, one or more samples in a replicate series revealed growth and microscopic examination of film preparations indicated close correspondence to the test organism, results were recorded as positive.

In the foregoing experiments, the spores were exposed to the quaternaries when suspended in fluids. Under conditions which usually prevail in food processing establishments, the spores may be contained in films of organic matter adhering to the surfaces of equipment. The capacity to reach and kill spores embedded in such films is an essential requirement of a chemical sterilizer. To obtain some information on the activity of quaternaries in these circumstances, spores were dried in skim milk films on small stainless steel strips (50 × 8 × 2 mm.). The sterile, fat-free strips first were dipped into cold milk heavily inoculated with spores, then suspended flatwise in a level plane on wood supports in

sterile petri plates and held at sub-minimum growth temperatures until the films were thoroughly dry, when the process was repeated two or three times.<sup>8</sup> The strips with adherent layers of dried milk film containing spores then were exposed to the quaternaries in buffer at pH 9.6 and 95° C. After the exposure, the strips were drained quickly and transferred to Lethen broth. One-tenth ml. quantities of the exposure substrate also were subcultured in separate flasks, since these usually contained some flakes of milk film detached from the strips during the heating treatment. Drying of the film is greatly retarded at low temperatures but at higher temperatures some of the mesophilic aerobic spores may germinate before drying of the films is complete.

Differences may be observed in the sporocidal activity of several compounds depending on the method by which they were tested (tables 3 and 4). Although the presence or absence of a protective film may be considered to be the chief variable, the numbers of spores exposed and subcultured were not directly comparable in the two tests. The results shown in table 4 indicate that, when the spores were protected in milk films, none of the quaternaries was able to kill all of the spores in all the test samples. This provides a rigorous test of effectiveness, yet the only safe criterion of the practical value of a sterilizing agent.

Since the germicidal activity of quaternaries may be enhanced by trisodium phosphate (18), some observations were made upon the sporocidal activity of quaternaries in this substrate. Dry milk films containing spores deposited on stainless steel strips as previously described were exposed to the quaternaries (1-20) at 95° C. in aqueous solutions of trisodium phosphate (2.22 per cent). The pH was unadjusted. As with the previously described strip results, the quaternaries did not consistently sterilize all the samples with exposures up to 30 min.

Many investigators have noted the powerful bacteriostatic action of quaternary ammonium compounds (1, 5, 6, 10, 19). Few have reported on the minimum concentration of quaternary required to produce sporostasis.

In tables 5 and 6 are given the approximate limiting concentrations of the compounds in nutrient broth and in skim milk. Since the amount of quaternary necessary to prevent development of the spores may be influenced by the number of spores present, some of the results are given at two levels of spore concentration. Viable vegetative cells were absent from most of the spore crops as harvested; in others, when prolonged incubation of the slopes did not eliminate the vegetative cells, the latter were killed by mild heating (85° C. for 15 min.) before the inoculations were made.

Table 5 shows that all the compounds (except the Amosols) were sporostatic in high dilution in nutrient broth. *B. metiens* was most tolerant, while certain strains of *B. subtilis* and *B. stearothermophilus* were inhibited by less than 1 part quaternary in 5 million.

Increasing the number of spores in broth usually increased the concentration of

<sup>8</sup> Concentration of viable spores in milk used to inoculate strips was approx. 100 million per ml. for *B. subtilis* cultures and 7 million per ml. for cultures 1518 and 3679. Each dip of strip picked up approx. 0.08 g. of fluid milk inoculum.

TABLE 5  
Concentration of quaternaries which produced sporostasis in nutrient broth

Culture	No. spores in 10 ml. medium	Roccal	Oxyside	Ceptryn	Nopocade K	Hyamine 10X	Hyamine 1652	Emcol 888	Tetrosan	Amosol #1 <sup>a</sup>	Amosol #2 <sup>a</sup>
		(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)	(p.p.m.)
<i>B. mycoides</i> 6462	50	1	1	1	1	0.4	1	1	1	40	40
	1,000,000	2	2	1	4	1	1	4	2		
<i>B. metiens</i>	50	2	2	2	2	2	4	4	2	200	1,000
	1,000,000	4	2	4	4	2	10	10	2		
<i>B. cereus</i> 401	50	1	2	1	2	1	1	2	1	40	40
	1,000,000	4	2	4	4	4	4	4	4		
<i>B. sphaericus</i> 4525	50	1	1	1	1	1	1	1	1	40	40
	1,000,000	2	2	2	2	2	4	2	1		
<i>B. circulans</i> 7049	50	1	1	1	1	1	1	1	1	40	40
	1,000,000	4	4	2	2	4	4	4	4		
<i>B. polymyza</i> 8523	50	2	2	1	1	2	2	2	1	100	100
	1,000,000	4	4	2	2	4	4	4	4		
<i>B. subtilis</i> LB	50	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	20	20
	1,000,000	< 0.4	< 0.4	< 0.4	1	< 0.4	< 0.4	< 0.4	< 0.4		
<i>B. subtilis</i> 6	50	2	1	1	1	1	1	1	1	40	40
	1,000,000	2	4	1	4	1	2	4	2		
<i>B. subtilis</i> 15u	50	< 0.1	0.2	0.4	0.4	0.1	0.4	0.4	1	20	20
	1,000,000	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4		
<i>B. atterimus</i> 230	50	1	1	1	2	1	1	1	1	40	40
	1,000,000	2	2	2	2	2	2	2	2		
<i>B. pumilus</i> 7061	50	1	1	1	1	1	1	1	1	40	40
	1,000,000	2	1	2	2	2	2	4	1		
<i>B. brevis</i> 8185	50	1	1	1	1	1	1	1	1	40	40
	1,000,000	2	4	2	4	2	4	4	2		
<i>B. steatothermophilus</i> 1518	50	1	0.2	0.2	1	1	1	1	1	> 1	> 1
<i>B. steatothermophilus</i> 26	50	< 0.1	< 0.1	< 0.1	0.2	< 0.1	0.2	0.2	1	> 1	> 1
<i>B. steatothermophilus</i> C <sub>2</sub> P <sub>2</sub>	50	0.2	< 0.1	< 0.1	0.2	0.2	0.2	0.1	1	0.2	0.2

<sup>a</sup> Data not directly comparable with that of the other compounds.



quaternary necessary to produce sporostasis; the greatly diminished sporostatic activity of quaternaries in milk is shown in table 6.

In this medium the minimal inhibiting concentration is frequently 1,000 times greater than for nutrient broth with comparable inocula.

#### DISCUSSION

It generally is recognized that surface film should be removed from equipment by prior detergent action to enable the sterilizing agent to act effectively; however, since this is not always realized, the performance of a germicide in the presence of organic matter is of practical significance.

The results of the foregoing study indicate that quaternary ammonium compounds are not efficient sterilizing agents against bacterial spores. Complete destruction of a spore population rarely can be effected by these compounds within 30 min. and then only at concentration levels and under other conditions of exposure that would greatly limit their commercial usefulness.

The conflicting nature of published reports in this field may be ascribed in small part to the use of different organisms but more particularly, to defects in the evaluation technique. Chief among these is the failure of many investigators to differentiate between sporocidal and sporostatic effects. Although quaternaries are strongly adsorbed on bacterial surfaces (22) and in many substrates may inhibit growth in high dilutions (*loc. cit.*), many have worked with these compounds in the relatively high concentrations necessary to affect spores, yet have made no provision for detoxification of the quaternary transferred with the spores to the subculture medium. In the present study, sporostasis was observed frequently in broth at dilutions so high that it would be impracticable to employ this method to eliminate the inhibiting effect. Of interest in this connection is the demonstration by Kivella *et al.* (15), that it is possible to remove surface-active cations from the surface of bacterial spores by a combination of dilution and vigorous shaking or centrifugation.

#### SUMMARY

Eight quaternary ammonium compounds and two quaternary-containing detergents were studied in respect to their spore-killing activity in buffer or distilled water solutions.

The sporocidal activity increased with temperatures at 30, 60, 80 and 95° C. The sporocidal activity increased with the degree of alkalinity at pH 6.4, 8.0 and 9.6.

Traces of skim milk and the protection afforded by milk films, in general, decreased the sporocidal activity of the quaternaries.

At the highest observed temperature (95° C.) and most favorable pH (9.6), concentrations of quaternaries up to 1-20 did not kill all the spores of all test samples within 30 min.

Quaternaries were highly sporostatic in nutrient broth and had relatively low sporostatic activity in skim milk.

Discrepancies in the reported sporocidal activity of quaternary ammonium compounds are discussed.

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# STUDIES ON THE FEEDING VALUE OF MOW-CURED BALED HAY<sup>1</sup>

W. A. KING,<sup>2</sup> J. W. WILBUR, S. M. HAUGE AND A. W. COOPER<sup>3</sup>

*Purdue University Agricultural Experiment Station, Lafayette, Indiana*

In the search for better methods of preserving high quality roughages much interest has been shown in the mow-curing of hay by both experiment station workers (2, 6, 11, 12, 13, 16, 20) and farmers. Most of the experimental work has centered around the completion of the drying of long or chopped hay and its feeding value. Fewer studies, however, have been made on the mow-curing of baled hay and its feeding qualities (6, 7, 14, 17).

Aitkenhead (1) in 1926 at the Purdue Agricultural Experiment Station seems to have been the first investigator in this country to use supplemental heat to aid in the curing of hay. In more recent years others (3, 6, 16, 17, 18) have applied heat with some success. Since in 1945 practically nothing was known about the mow-curing of baled hay and because field balers were being developed rapidly, a study on the feeding value of mow-cured baled hay was undertaken.

## PROCEDURE

The feeding value of field-cured and mow-cured baled alfalfa hays was compared in feeding trials conducted during the winter months for a period of 3 successive years. In order to eliminate the factor of different soil conditions, the hays studied were produced on the same field and alternating windrows were used for the field and the mow-cured hays. All hays except the first cutting in the third year were cut between the one-half and three-quarter bloom stage.

After cutting, the hays for mow-curing were partly dried in the field to a moisture content ranging from 30 to 40 per cent, whereas the field-cured hays were dried to about 20 per cent moisture content. All hays were baled in the field with a pickup baler and hauled immediately to the barn. The hays to be mow-cured were placed in a 20 × 20 × 10 ft. high experimental drying bin with a slatted floor, and air, at the rate of about 25 ft.<sup>3</sup> per min. per ft.<sup>2</sup> of floor space, was forced through the hay until cured. In the first 2 yr. unheated air was used, whereas heated air was used in the third year (8.5 and 15° F. rise). The first cutting hays each year contained considerable timothy, while the other cuttings were almost pure alfalfa.

In the first 2 yr. three cuttings of alfalfa hay were studied; two cuttings were used in the third year. The reason for the difference in the third year was that excessive rains in the fore part of June delayed the first cutting until the last week

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<sup>2</sup>Present Address: Dairy Department, The Clemson Agricultural College, Clemson, South Carolina

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of that month. Therefore, only two cuttings were obtained that year. Each of the cuttings of all 3 yr. was fed to dairy cows in test periods of 9 wk.

The groups fed the experimental hays included five to six cows each and consisted of Holsteins, Jerseys, and Guernseys, with the Holsteins predominating. The two groups of cows of each experiment were balanced as evenly as possible at the time of allotment. The hays were fed to the experimental cows as the only roughage at the rate of 2.5 lb. of hay per 100 lb. of live weight. This proved to be about all the hay the cows would consume. A 13.5 per cent total protein grain mixture was fed to the cows on the basis of 1 lb. of grain for each 4 lb. of 4 per cent fat-corrected milk produced by the cows in a preliminary period before each feeding trial. The grain mixture consisted of 400 lb. yellow corn, 200 lb. oats, 100 lb. linseed oil meal, 10 lb. iodized salt and 7 lb. steamed bone meal.

The carotene contents of the hays were determined by a modification of the method of Moore and Ely (19), of the blood plasma by Moore's method (10) and of the butterfat by the method of Koehn and Sherman (8). The butterfat was assayed for vitamin A potency by the usual rat-growth method, using USP reference oil as a standard.

#### RESULTS AND DISCUSSION

The carotene content of the hays was used as a sensitive index to the state of hay preservation. Table 1 contains the carotene values of the field- and mow-

TABLE 1  
*Carotene content and grades of the experimental baled alfalfa hays*

Yr.	Cutting	Carotene (ppm on dry basis)				Federal grade no. <sup>b</sup>
		As cut	Into barn	Cured	As fed	
1945	1st.—Field	209	67 <sup>a</sup>	24	10	
	Mow	209	101	30	13	
	2nd.—Field	226	48 <sup>a</sup>	15	9	
	Mow	226	93	56	25	
	3rd.—Field	216	48	31	25	
	Mow	216	52	42	25	
1946	1st.—Field	167	116	26	3	3
	Mow	167	107	27	7	2
	2nd.—Field	303	7 <sup>a</sup>	3	2	3, leafy
	Mow	303	85	25	15	1, extra leafy
	3rd.—Field	210	23 <sup>a</sup>		12	1
	Mow	210	59		39	1, extra leafy, extra green
1947	1st.—Field	150	9 <sup>a</sup>	9	4	2
	Mow	150	16 <sup>a</sup>	10	7	1
	2nd.—Field	360	93	15	5	3, leafy
	Mow	360	144	76	48	1, extra leafy, extra green

<sup>a</sup> Rain on the hay in the field.

<sup>b</sup> No Federal grade available in 1945

cured baled hays by each year's cuttings as cut, as going into the barn, as completely cured (after barn drying) and as fed some 5 to 6 mo. later. In table 1, the carotene content also may be compared to official federal gradings of the experimental hays. As a rule, the mow-cured hays graded at least one grade higher than the field cured.

It is very interesting to note the difference in the rate of carotene destruction after the forage was cut and up until the time of storage. This destruction in the field was much more rapid in the case of the second and third cutting hays of the first 2 yr. than the first cutting. One logical explanation for this is the lower temperatures and more hours of cloudiness which would retard the destructive action of the enzyme lipoxidase (9). Any advantage in the lower carotene destruction of the first cutting hays in the field was lost, however, in storage. In all 3 yr. of study, the carotene content of the first cutting hays, regardless of treatment, were relatively low at the time of feeding. This has been observed by others (11). Even heated air (8.5° F. rise) in the third year was of no obvious benefit in curing the first-cutting mow-cured hay. This, however, was due to the facts that the latter hay was past bloom stage and, therefore, relatively low in carotene at the time of cutting and also that most of the carotene was lost in the field before mow-curing.

One of the real advantages of mow-curing of hay is the early removal of the hay from the field without much, if any, rain on it. This is illustrated by the figures in table 1 where rain on the various hays is indicated and the corresponding greater carotene destruction can be noted. In four out of eight sets of experimental hays the mow-curing process allowed the hay to be placed in the barn without rain, whereas, the corresponding field-cured hays were rained on. In only one out of eight cases did rain fall on both the field- and mow-cured hays. Rain does the greatest damage when it falls on hay after the hay is partly dry.

The mow-curing process without supplemental heat showed its greatest advantage in the second cutting hays when the outside air temperature was high and not very humid but showers were rather frequent. Even so, the over-all differences observed in this study between mow- and field-curing are not striking. It appears, then, from the losses in carotene content of the hays that the mow-curing of baled hay with unheated air, using about 25 ft.<sup>3</sup> of air per min. per ft.<sup>2</sup> of floor space, is difficult and, at best, the results are likely to be quite variable. The application of heat (15° F. rise) in the third year, second cutting, proved to be very successful and appears to offer a much more dependable process for baled hay. From studies by other experiment station workers (5, 12, 13, 20, 21) on the mow-curing of long hay with unheated air, the carotene content of long hay seems to be more easily preserved and, undoubtedly, a higher quality hay usually results than is the case of baled hay dried with unheated air.

In general, the carotene content of the experimental hays is reflected directly in the cows by the carotene content of the cows' blood and butterfat and the vitamin A potency of the butterfat (table 2). The carotene content of the bloods was determined every 3 wk. during the trial. The carotene and the vitamin A potency of composite samples of butterfat were determined also at the end of each 9-wk. feeding trial. The carotene contents of the hays were reflected in the carotene contents of the blood, the milk fat and the vitamin A potency of the milk fat during the successive periods of each trial.

A summary of each 9-wk. feeding trial for the 3 yr. is presented in table 3.

TABLE 2  
*The carotene content of cows' blood and milk as influenced by feeding baled alfalfa hays*

	First cutting						Second cutting						Third cutting					
	1945		1946		1947		1945		1946		1947		1945		1946		1947	
	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow
Carotene in hay (ppm/D.M.)	10	13	3	7	4	7	9	25	2	15	5	48	25	25	12	39		
Av. carotene intake (mg./d.)	111	144	33	77	53	92	101	296	24	184	67	620	304	302	152	497		
Blood carotene* (γ/ml.)																		
Initial	7.6	7.5	7.8	8.3	6.6	8.7	2.4	2.7	5.6	5.1	3.4	2.6	3.0	2.4	4.3	1.3		
3 wk.	3.4	3.0	6.6	6.4	3.7	5.0	1.8	3.6	2.3	4.8	1.9	3.7	3.3	2.7	4.2	4.7		
6 wk.	2.7	3.3	6.0	5.5	2.3	3.2	1.7	3.3	1.4	4.6	1.0	3.3	2.8	2.5	3.2	4.1		
9 wk.	2.4	3.0	4.3	4.5	1.8	2.6	1.3	2.8	1.3	4.3	1.6	4.3	2.7	2.4	3.7	4.8		
Milk carotene (γ/g. fat)																		
Initial			6.6	6.0	5.6	6.0	3.8	3.5	4.1	5.6	1.8	1.8	2.4	3.2	2.8	1.0		
Final	2.4	3.2	3.4	3.9	1.9	1.3	2.2	3.5	1.0	2.8	1.0	2.8	3.8	3.5	2.3	3.4		
Vitamin A (I.U./g. fat)																		
Initial			47	38	28	27	35	37	38	40	16	15	27	28	22	15		
Final	27	28	30	35	17	14	25	40	15	22	9	Lost	36	37	22	40		

\* 1945—whole blood; 1946 & 1947—plasma.

TABLE 3  
Summary of 9-wk. hay feeding trials (lb.)

	First cutting				Second cutting				Third cutting			
	1945		1946		1947		1945		1946		1947	
	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow
No. of cows	6	6	6	6	5	5	6	6	6	6	5	5
Grain consumed	9.1	9.1	7.8	7.8	9.0	9.3	7.0	7.0	7.7	7.8	6.0	6.1
Hay consumed	24.6	24.2	26.0	26.0	29.3	28.9	26.4	26.5	28.5	28.7	29.7	30.3
Av. wt. of cows	1003	1024	1130	1139	1227	1277	1081	1062	1200	1213	1193	1231
Av. gain in wt.	59	57	20	25	24	32	29	46	19	40	34	18
Av. daily milk	28.7	27.4	24.2	24.4	26.7	25.4	22.9	23.5	27.1	27.0	22.0	24.7
Av. daily 4% F.C.M. <sup>a</sup>	29.6	28.6	25.2	25.7	27.2	27.0	23.5	24.6	27.4	28.4	23.5	25.2
Av. decline 4% F.C.M.	10.7	7.9	3.5	3.9	8.8	14.6	2.9	2.6	3.0	3.9	4.0	0.1
Av. 4% F.C.M. produced per lb. T.D.N. consumed	1.47	1.44	1.33	1.36	1.27	1.26	1.18	1.22	1.37	1.40	1.16	1.22
											1.09	1.05
											1.19	1.20

<sup>a</sup>Gaines' formula used (4).

Since the groups of cows from one experiment to another were not necessarily similar, no detailed comparisons should be made from year to year or cutting to cutting. Only the field- and mow-cured hays of the same cutting and year can be compared directly. Although none of the possible comparisons of the value of the hays in table 3 showed significant differences, it is interesting to note two things. In five out of eight trials the cows fed the mow-cured hays produced a small amount more milk than those fed the field-cured. Also, in the case of the second cutting hay, mow-cured in the third year by means of heated air, the cows showed remarkable persistency. Of the five cows in this group, three were producing more milk at the end of the 9-wk. feeding trial than at the beginning, one held her production and one dropped, giving an average decline of only 0.1 lb. of 4 per cent fat-corrected milk for the entire period. The cows in the experiments were fed controlled amounts of hay (2.5 lb. of 100 lb. live weight) and grain. For this reason large differences in the feeding value of the hays would have to exist in order to demonstrate significance.

On the whole, the observations on the feeding value of the experimental hays are not greatly different from those found at other experiment stations. Rollins and Reaves (13) found in a double reversal feeding trial that 4 per cent more milk was produced by the cows fed barn-cured hay as compared to those fed field-cured hay. Morrison and Turk (11), in a summary of 3 yr. of studies, found no significant difference between the barn-cured and field-cured hays as measured by daily milk production and hay consumption. Also, in experiments by Shepherd *et al.* (15, 16) no significant difference was found between the average daily milk production of cows fed barn-cured and field-cured hays.

When equal amounts of hays with similar leaf content and from the same crop are fed, the above results on milk production might well be expected, because from the work by Camburn (2) the digestible protein and total digestible nutrients are about the same for similar hays cured either in the barn or field. Concluding from all observations it would seem that the mow-cured hay probably would have to average at least two grades above that of the field-cured in order to show significant differences between mow- and field-curing processes.

It is only when the mow-curing system consistently produces high quality hay and the field-cured hay has been abused by leaching rains and/or by improper handling for best preservation of hay that significant differences on a pound for pound basis can be expected. In such cases of wide differences between hays cured in the barn or field, palatability also would become a factor in hay feeding experiments. The importance of palatability can be shown more satisfactorily in trials where the hay is fed *ad libitum*.

Another way in which mow-curing systems show up to an advantage is the nutrients preserved per acre. Work by Shepherd *et al.* (5, 15, 16) has shown striking advantages for preservation of hay crops as wilted silage, as compared to field- or mow-cured hay. Again, mow-cured hay showed a distinct advantage over field-cured hay when measured on the basis of the preservation of the original plant nutrients in the field at the time of cutting. However, carotene

was the only nutrient whose losses during the curing processes and storage was studied in our experiments.

#### SUMMARY

The feeding value of similar baled alfalfa hays cured either in the mow or field was studied over a period of 3 yr. Without the aid of heated air the carotene content of the mow-cured hays was variable, depending on the prevailing weather conditions. When the outside air was cool and humid, as in the cases of the first-cutting hays, the mow drying process was prolonged, with most of the carotene in the hay being destroyed.

The mow-curing of baled hay seems to be a much more difficult process than the curing of long hay because of the difficulty of getting air to pass through the bales, even though loosely packed. The use of supplemental heat is to be recommended as a more dependable processing procedure for baled hay.

When compared in feeding trials in which equal quantities of hays were fed on a cow-weight basis and with concentrates at the rate of 1 lb. for each 4 lb. of 4 per cent fat-corrected milk, no significant differences in feeding value were found between baled hays cured either in the mow or in the field. The mow-cured baled hays seemed to be as palatable to the dairy cows as the field-cured baled hays.

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# A SYNTHETIC PABULUM VS. YOLK-CITRATE BUFFER AS A DILUTER OF BULL SEMEN<sup>1</sup>

N. D. BAYLEY, H. V. CORBS AND G. R. BARRETT<sup>2</sup>

*University of Wisconsin, Madison*

In 1939, Phillips (4) developed an egg-yolk buffer for preserving and diluting bull semen. That this buffer was satisfactory for use in artificial breeding was indicated by Phillips and Lardy (5) with limited field trial data. Salisbury, Fuller and Willett (7) reported advantages in substituting sodium citrate for the dibasic sodium phosphate in Phillips' buffer. Use of the yolk-phosphate or the yolk-citrate buffer has become a general practice in artificial breeding.

In 1946, Phillips and Spitzer (6) developed a synthetic pabulum. The essential ingredients were "freshly purified lipids, specific sugars—glucose and galactose—a buffer system, a gum to supply the proper physical consistency and an agent to control bacterial contamination." Limited field trial data indicated that breeding results from the use of such a pabulum might be comparable to those from the use of yolk-buffer.

The object of the investigation reported here was to obtain further information concerning the fertility of semen diluted with this synthetic pabulum<sup>3</sup> as compared with semen diluted with yolk-citrate buffer.

## EXPERIMENTAL PROCEDURE

The dry ingredients of the synthetic pabulum were prepared in two compounds by the Department of Biochemistry in accordance with the formula published by Phillips and Spitzer (6). Once each week the compounds were combined in solution for use. Twenty-five g. of compound No. 1 were added to 50 ml. of distilled water at boiling temperature and stirred until the solute was dissolved. Three and one-half g. of compound No. 2 were added and dissolved as completely as possible. The solution then was cooled and stored at a temperature of 40 to 42° F.

The yolk-citrate buffer was prepared twice a week. Thirty-two g. of crystalline  $\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  were dissolved in 1000 ml. distilled water and brought to boiling. After cooling, this solution was mixed with an equal volume of egg yolk, strained and stored at a temperature of 40 to 42° F.

This field trial employed semen from ten Holstein and nine Guernsey bulls in the University of Wisconsin bull stud. Their 60- to 90-day non-returns for the period of the experiment ranged from 23 to 68 per cent.

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Semen was collected on Monday and Thursday of each week and was used routinely for 3 days. No experimental breedings were made on Sunday. A system of balancing was carried out so that, as far as possible, semen collections were made from all 19 bulls with equal frequency.

Immediately after collection, the semen was examined for motility and diluted 1:30 to 1:40, depending on the volume collected and the amount of diluted semen needed. Both diluters were warmed in a water bath to 65 to 70° F. prior to being mixed with raw semen. One-half of each semen sample was diluted with the synthetic pabulum and the other half with yolk citrate. The rate of dilution was the same for both treatments.

Inseminations for this experiment were made during the period from May 3 to September 13, 1948. Fertility was measured by pregnancy examinations (8) and was based on both first and second services in herds in Dane County. The two treatments of semen were used alternately during the day so that the same number of inseminations per treatment were made insofar as the number of cows to be bred permitted.

TABLE 1

*Breedings with semen from bulls having 60- to 90-day non-returns below 40 per cent for 2 or more mo. during the period of the experiment*

Day of use	Treatment	No of breedings	% Fertile <sup>b</sup>	Chi-square
1 <sup>a</sup>	Synthetic pabulum	71	14.1	0.06
	Yolk citrate	71	12.7	
2	Synthetic pabulum	75	9.3	2.08
	Yolk citrate	75	17.3	
3	Synthetic pabulum	64	6.2	4.58 <sup>c</sup>
	Yolk citrate	64	18.8	
Over all 3 d. of use	Synthetic pabulum	210	10.0	3.54
	Yolk citrate	210	16.2	

<sup>a</sup> Day of use 1 is the day of collection.

<sup>b</sup> These fertility percentages are based on pregnancy examinations. The same results based on 60- to 90-d. non-returns would be approximately 6 to 7 % higher. (1)

<sup>c</sup>  $P < 0.05$ .

TABLE 2

*Breedings with semen from bulls having 60- to 90-day non-returns above 40 per cent*

Day of use	Treatment	No. of breedings	% Fertile <sup>b</sup>	Chi-square
1 <sup>a</sup>	Synthetic pabulum	144	38.2	10.50 <sup>c</sup>
	Yolk citrate	144	57.6	
2	Synthetic pabulum	146	31.5	11.88 <sup>c</sup>
	Yolk citrate	146	51.4	
3	Synthetic pabulum	142	29.6	14.04 <sup>c</sup>
	Yolk citrate	142	51.4	
Over-all 3 d. of use	Synthetic pabulum	432	33.1	36.82 <sup>c</sup>
	Yolk citrate	432	53.5	

<sup>a</sup> Day of use 1 is the day of collection.

<sup>b</sup> These fertility percentages are based on pregnancy examinations. The same results based on 60- to 90-d. non-returns would be approximately 6 to 7 % higher. (1)

<sup>c</sup>  $P < 0.01$ .

Inseminations where the semen was deposited in the cervix instead of the uterus were eliminated. The daily first-service inseminations made by each inseminator and with semen of each bull were balanced so that there were an equal number of breedings for each treatment. The daily second-service breedings were balanced in the same manner. Such elimination of breedings as was found necessary was carried out by the use of random numbers. The data were then tabulated and analyzed by chi-square.

The trial was planned to terminate when a preliminary analysis of the data indicated a real difference in fertility between the treatments of not less than 5 per cent.

#### RESULTS AND DISCUSSION

The data obtained are presented in tables 1 and 2. The fertility of semen diluted with yolk-citrate averaged 15 per cent above the fertility of semen diluted with the synthetic pabulum. The chi-square test showed this difference to be highly significant. Allowing for the error that one would expect in the average difference found in this trial, the real difference may be considered as lying within the range 7 to 23 per cent.<sup>4</sup> The interaction chi-square (3) between days of use was not significant, indicating that the difference between treatments may be considered the same for all days of use.

Bulls whose 60- to 90-day non-returns were lower than 40 per cent for 2 mo. or more during the experiment were considered as "bulls of relatively low fertility." The results of experimental breedings made with semen from these bulls are shown in table 1. Results of breedings made with bulls of "relatively high fertility" are shown in table 2. The tables appear to show that the difference between diluters was greater for the bulls of higher fertility than for the bulls of lower fertility. However, the statistical analysis did not substantiate this. The analysis also indicated that the results of the trial did not vary significantly between individual bulls, between breeds or between inseminators or services.

In drawing conclusions regarding the level of fertility shown here as resulting from the use of this synthetic pabulum, three considerations should be made: (a) The level of fertility found with this diluter depended to some extent on the fertility level of the bulls used. (b) The fertility percentages shown here are based on pregnancy examinations and would be approximately 6 to 7 per cent higher if 60- to 90-day non-returns had been used (1). (c) The comparison between the two diluters is more important in this trial than is the actual fertility level obtained with the synthetic pabulum.

#### SUMMARY

In a trial involving 1,284 field inseminations, the fertility was 15 per cent higher with semen of 19 bulls diluted with egg-yolk citrate buffer than with other portions of the same semen diluted with a synthetic pabulum. The real difference may be considered as lying within the range 7 to 23 per cent.

<sup>4</sup> 95% fiducial limits (2).

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# THE INFLUENCE OF CRACKED SOYBEANS, SOYBEAN HAY AND VARIOUS KINDS OF CONTAINERS ON THE FLAVOR OF MILK<sup>1</sup>

E. E. BARTLEY,<sup>2</sup> J. W. F. CHIN AND C. Y. CANNON

*Dairy Husbandry Subsection*

AND

E. W. BIRD

*Dairy Industry Section*

*Iowa Agricultural Experiment Station, Ames*

The Iowa dairy industry long has been troubled with the development of oxidized and other undesirable flavors in milk. Since soybeans are the chief source of home-grown protein in Iowa, some of these flavors were attributed to soybean feeding. Creamery operators of the state continually reported that they were receiving milk and cream which had what was described as a "soybean" flavor.

Earlier work at this station (4, 5, 6, 12, 13) showed no indications of milk off-flavors when soybeans were fed in the usual amounts. Similar results were obtained by other workers (2, 7, 8, 9, 10). However, since soybeans have been indicated to increase the proportion of unsaturated fatty acids in butterfat, and since a number of farmers occasionally transport their milk in rusty tin cans, it was felt that the exposed surface of the iron might act as a catalyst aiding in the oxidation of the unsaturated fatty acids, thus resulting in oxidized flavors.

An experiment was set up to compare the milk from a group of cows fed cracked soybeans in amounts usually used by Iowa dairymen with that of another group fed similar amounts of linseed oil meal. The milk from both groups was collected in glass, tinned iron and rusty tinned iron containers<sup>3</sup> and scored for flavor.

At the conclusion of this experiment, a second experiment was initiated to answer certain questions raised by the results of the first. Since the first experiment was conducted during the months of April to July, it was felt that work on similar lines should be conducted during the cold months,<sup>4</sup> as there might be a seasonal effect involved. The effect of soybean hay was not studied in the previous work. Therefore, in the second experiment, the effect of soybean hay on the flavor of milk was checked simultaneously with that of cracked soybeans.

## EXPERIMENT I

*Method of experimentation.* Previous work (4) has shown that when selecting cows for flavor studies, selection should involve the flavor scores of their

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<sup>2</sup> Present address: Kansas State College, Manhattan.

<sup>3</sup> For brevity the tinned iron and rusty-tinned iron containers will be referred to subsequently as tin and rusty tin containers, respectively.

<sup>4</sup> The second experiment extended from Nov. through May.

milk. Sixteen Holstein cows were chosen from the college herd and fed a common ration for a preliminary period of 24 days. During this period individual milk samples were collected and scored twice a week for flavor.

On the termination of the preliminary period 10 of the 16 cows were selected and paired as similarly as possible with regard to age, stage of lactation, production of milk and butterfat and the flavor scores of their milk. The cows in each pair then were placed at random in one or the other of two groups.

A double reversal design of feeding was used (table 1) comparing two rations

TABLE 1  
*Feeding schedule—Experiment I*

	Duration (d.)	Rations fed to:	
		Group I	Group II
Preliminary Period	24	Standard ration	Standard ration
Transitional period	10	Soybean ration	Linseed oil meal ration
Exp. period I	21		
Transitional period	10	Linseed oil meal ration	Soybean ration
Exp. period II	14		
Transitional period	10	Soybean ration	Linseed oil meal ration
Exp. period III	11		
Transitional period	10	Linseed oil meal ration	Soybean ration
Exp. period IV	13		

which were similar except that the experimental concentrate contained 11.1 per cent cracked soybeans, whereas the control contained an equivalent amount of linseed oil meal substituted for the soybeans. The grain rations contained four parts corn, two parts rolled oats, two parts wheat bran, and one part cracked soybeans or linseed oil meal. This ration was fed at the rate of 1 lb. grain to every 4 lb. of milk produced. Alfalfa hay of fair quality was fed *ad libitum* as the only roughage. A 10-day transitional period was allowed whenever the feeds were reversed.

Milk samples were collected for scoring three times during each feeding period. When sampling, portions of each cow's milk were put in a 0.5-pt. milk bottle, a 1-pt. tin container and a 1-pt. rusty tin container. The rusty tin containers were made as uniformly rusty as possible by scratching one line deeply around the side and two lines across the bottom of the tin plated surface. The untarnished tin containers immediately were replaced by new ones, once any corrosion in them was noticed. All containers were sterilized in an autoclave before being used.

The milk samples were gathered at 3:30 A.M. on collection days and cooled immediately. They were scored about 12 hr. later by experienced judges from the Dairy Industry Department. The score card suggested by the Committee on Score Cards, A.D.S.A. (1, p. 4) was used.

*Results and discussion.* The flavor score data showing differences between the two rations and differences between the three types of containers are summarized in table 2. The differences in scores of the milk produced by the two

TABLE 2

*Average flavor scores of milk held in glass, tin and rusty tin containers for each group during each sampling when the animals were fed either cracked soybeans or linseed oil meal. Experiment I*

Feeding periods	Samplings	Soybean ration				Linseed oil meal ration			
		Group	Containers			Group	Containers		
			Glass	Tin	Rusty tin		Glass	Tin	Rusty tin
I	1	I	37.9 <sup>a</sup>	37.7	37.5	II	37.9	37.6	37.5
	2		37.2	37.2	37.1		37.3	37.6	37.9
	3		37.2	37.5	37.9		37.4	37.9	37.7
II	1	II	37.6	37.7	37.9	I	37.9	37.6	37.8
	2		37.8	37.9	38.0		37.8	37.8	37.5
	3		37.6	37.7	37.8		37.9	38.0	37.8
III	1	I	38.2	38.1	38.0	II	38.0	37.9	38.1
	2		37.9	38.0	38.0		38.0	38.0	37.8
	3		38.2	38.0	37.9		37.8	38.0	37.8
IV	1	II	37.3	37.2	37.3	I	37.5	37.5	37.3
	2		38.0	37.9	37.9		38.1	38.0	37.9
	3		37.9	37.9	37.9		38.0	38.2	37.8
Container Av.			37.73	37.73	37.77		37.80	37.84	37.74
Ration Av.			37.74			37.79			

\* Each of these scores is an av. of five scores obtained from the five cows in each group.

rations were so slight that, under the conditions of this trial, soybeans did not produce flavors in milk that were more undesirable than those produced when linseed meal was the concentrate. No oxidized flavors developed in the milk during the experiment, even though no green feed was fed. Little difference in quality occurred in the milk collected and held for a 12-hr. period in glass, in tin and in rusty tin containers.

#### EXPERIMENT II

*Method of experimentation.* Sixteen Holstein cows were fed a common ration for a preliminary period of 15 days. At the end of this period 12 cows were selected and placed in three groups as uniformly as possible with regard to flavor of milk, number of previous lactations, stage of lactation, size and production level. A cow from each of the three groups was started on one of the following four experimental rations: ration A—soybean hay as roughage, a basal grain mixture and 11.1 percent linseed meal as the protein supplement; ration B—alfalfa hay as roughage, a basal grain mixture and 11.1 percent cracked soybeans as the protein supplement; ration C—soybean hay as roughage, a basal grain mixture and 11.1 percent cracked soybeans as the protein supplement; and ration D—alfalfa hay as roughage, a basal grain mixture and 11.1 percent linseed meal as the protein supplement. Hay was fed *ad libitum*; grain was fed at the rate of 1 lb. for every 4 lb. of milk produced.

Since four rations could not be tested adequately during the lactation period through the double reversal design, the design suggested by Cochran, *et al.* (3) was used. This design (table 3) reduces variation resulting from cow and ra-

TABLE 3  
*Feeding schedule—Experiment II*

	Dura- tion	Rations fed											
		Group I				Group II				Group III			
		Cow no.				Cow no.				Cow no.			
		2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional period Exper. Period I	(d.) 14 20	A	B	C	D	A	B	C	D	A	B	C	D
		2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional period Exper. Period II	14 17	B	A	D	C	D	C	B	A	C	D	A	B
		2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional period Exper. Period III	14 18	C	D	A	B	B	A	D	C	D	C	B	A
		2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional period Exper. Period IV	14 21	D	C	B	A	C	D	A	B	B	A	D	C

tion differences, progress of lactation and seasonal change. Each group of four cows constitutes an independent experiment designated as a  $4 \times 4$  Latin square. The important features of the design are: (a) all rations are received by each group during every period, (b) each ration is followed by a different ration from one period to the next and (c) each cow receives a different permutation of rations during the four periods. The cows were kept on a transitional period of 14 days prior to each test period. The schedule of feeding is shown in table 3.

The milk samples for scoring collected from each cow twice each week were held in 0.5-pt. milk bottles, 1-pt. tin containers and 1-pt. rusty tin containers. Samples were iced and held about 21 hr. before they were scored. The score card (11, p. 567) was essentially the same as the one used in the first experiment, except that it was more specific in that the intensities of the various flavors were given definite scores.

*Results and discussion.* Milk samples were collected twice each week from each cow. This procedure yielded six samples (and six scores) for each cow and each container type during each experimental period of the study.

The average flavor scores of the six samples of each cow's milk (for all container types) are shown in table 4 for each of the experimental periods. The scores obtained for the milk held in glass were analyzed statistically for variance;



TABLE 4  
Average flavor scores of milk held in glass, tin and rusty tin containers for each cow during each period

Period	Group I				Group II				Group III			
	Cow. no.		Cow. no.		Cow. no.		Cow. no.		Cow. no.		Cow. no.	
	2346	2344	2335	2310	2378	2370	2214	2210	2197	2000	1566	1297
Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration
Glass containers												
I	A 35.8	B 36.5	C 37.5	D 35.7	A 36.6	B 36.1	C 36.4	D 35.8	A 37.2	B 35.1	C 37.4	D 36.1
II	B 36.0	A 36.3	D 36.2	C 36.3	D 36.1	C 36.8	B 35.3	A 36.1	C 36.7	D 35.9	A 36.9	B 36.3
III	C 35.7	D 35.8	A 36.0	B 35.0	B 35.5	A 35.4	D 34.9	C 35.9	D 35.7	C 35.9	B 36.3	A 35.7
IV	D 35.0	C 36.0	B 35.4	A 36.1	C 36.5	D 35.5	A 34.5	B 35.3	B 33.5	A 35.8	D 35.7	C 36.3
Tin containers												
I	A 35.9	B 36.6	C 36.2	D 36.2	A 36.8	B 36.2	C 36.4	D 35.6	A 35.5	B 35.3	C 37.3	D 35.8
II	B 35.9	A 36.4	D 36.0	C 36.5	D 36.3	C 36.7	B 35.4	A 36.8	C 37.0	D 37.0	A 36.4	B 36.4
III	C 35.6	D 35.7	A 35.8	B 35.5	B 36.2	A 36.4	D 34.9	C 35.9	D 35.6	C 36.2	B 36.3	A 36.5
IV	D 35.3	C 36.5	B 34.9	A 35.7	C 36.8	D 35.3	A 34.9	B 35.7	B 36.0	A 36.0	D 35.9	C 36.3
Rusty tin containers												
I	A 35.7	B 36.8	C 36.2	D 34.8	A 35.5	B 36.5	C 35.6	D 34.3	A 34.7	B 34.7	C 36.1	D 34.1
II	B 35.8	A 36.1	D 35.9	C 36.3	D 36.1	C 36.7	B 35.7	A 35.7	C 36.8	D 37.0	A 36.1	B 36.0
III	C 36.0	D 35.4	A 35.4	B 35.3	B 35.3	A 35.8	D 34.5	C 35.5	D 35.8	C 36.3	B 36.1	A 35.2
IV	D 35.0	C 36.3	B 35.3	A 35.9	C 36.5	D 35.2	A 34.8	B 35.0	B 33.5	A 36.0	D 35.7	C 35.6

the results are presented in table 5. The scores obtained for milk held in tin

TABLE 5

*Analysis of variance of the flavor scores of milk held in glass containers*

	Degrees of freedom	Sum of squares	Mean squares
Between groups	2	16.9	8.45
Between cows within groups	9	216.3	24.0 *
Between periods within groups	9	232.5	25.83**
Between rations	3	212.8	70.9 **
Ration X group interactions	6	45.1	7.5
Error	19	130.05	6.8
Total	48	854.25	7.0

\* Significant ( $p < 0.05$ ).

\*\* Highly significant ( $p < 0.01$ ).

and in rusty tin containers were subjected to similar statistical treatment. The results are presented in tables 6 and 7. The "ration X group interactions"

TABLE 6

*Analysis of variance of the flavor scores of milk held in tin containers*

	Degrees of freedom	Sum of squares	Mean squares
Between groups	2	7.0	3.5
Between cows within groups	9	220.9	24.5*
Between periods within groups	9	162.9	18.1
Between rations	3	156.3	52.1**
Ration X group interactions	6	56.14	9.3
Error	19	145.1	7.6
Total	48	748.3	8.04

\* Significant.

\*\* Highly significant.

TABLE 7

*Analysis of variance of the flavor scores of milk held in rusty tin containers*

	Degrees of freedom	Sum of squares	Mean squares
Between groups	2	13.3	6.6
Between cows within groups	9	222.3	24.7
Between periods within groups	9	177.7	19.7
Between rations	3	160.0	53.3*
Ration X group interactions	6	62.1	10.3
Error	19	250.3	13.1
Total	48	885.7	12.4

\* Significant.

in all these tables are insignificant and therefore, are included in the experimental error.

The mean square of the ration effects is significantly greater than the error mean square in all three analyses of variance tables, indicating that there are differences between the rations in their effect on milk flavor. The average scores

for each ration in each container (table 8) show that the general trends in the

TABLE 8

*Average flavor scores of milk for the different rations when the milk was held in glass, tin and rusty tin containers*

Rations	Av. flavor score of milk held in:		
	Glass	Tin	Rusty tin
C. Soybean hay + cracked soybeans	36.4	36.4	36.1
A. Soybean hay + linseed meal	36.0	36.1	35.6
D. Alfalfa hay + linseed meal	35.7	35.8	35.3
B. Alfalfa hay + cracked soybeans	35.5	35.7	35.5

effects of the rations are almost identical for each of the types of containers. Ration C received the highest flavor score in all three containers. Ration A received the second highest score in all three containers. Ration D received the third highest score in the glass and tin containers and the lowest score in the rusty tin container. Ration B received the lowest score in the glass and tin containers and the third highest score in the rusty tin container.

The *t*-test indicated a highly significant difference between ration C (soybean hay and cracked soybeans) and ration A (soybean hay and linseed meal), a significant difference between ration A and ration D (alfalfa hay and linseed meal) and a non-significant difference between ration D and ration B (alfalfa hay and cracked soybeans).

Milk produced on rations B and D, both of which contained alfalfa hay, received the lowest flavor scores in all three kinds of containers, while milk produced on A and C, which contained soybean hay, received the highest scores in all three containers. Probably the differences between rations result from differences between the hays. The protein supplements do not seem to be responsible because the milk which received both the highest and lowest flavor scores was produced on rations containing cracked soybeans, while the rations that contained linseed meal produced milk with scores between those received by milk produced on the cracked-soybean rations.

The data in table 8 indicate that the effect of containers has the same general trend with all rations. The milk in the tin containers scored highest. This was followed in order by the milk in glass and in rusty tin containers. The *t*-test indicated no significant differences between the glass and tin containers but a significant difference between the tin and rusty tin containers. Apparently, tin containers free from rust are as satisfactory as glass containers, but rusty tin containers cause deterioration of milk flavor.

Oxidized flavors occurred 77 times in the 864 samples. These flavors were divided among the rations as follows: 21 occurred in the milk from the cows receiving ration A, 17 in the milk from those receiving ration B, 14 in the milk from those receiving ration C and 25 in the milk from those receiving ration D. Chi-square was computed using a  $4 \times 2$  table. The differences in occurrence of oxidized flavors could not be attributed to differences in rations.

The oxidized flavors were divided among the containers as follows: 12 occurred in glass, 9 in tin and 56 in rusty tin containers. This difference is highly significant, showing that the rusty tin containers tend to increase the susceptibility of the milk to oxidation. Therefore, results in experiment II are not in agreement with those from experiment I, in which the quality of the milk was not influenced by the type of container. This difference may have resulted from the longer time that the milk was held before scoring in experiment II or to seasonal influences, since experiment II was conducted during the winter months.

As the experiment progressed, unclean flavors became apparent in the milk (table 9). The increase in the occurrence of unclean flavors from the first two

TABLE 9  
*Occurrence of unclean flavors in milk: per ration, per period*

Periods	A Soybean hay + linseed meal	C Soybean hay + cracked soybean	B Alfalfa hay + cracked soybean	D Alfalfa hay + linseed meal	Total
1	3	3	4	5	15
2	1	--	2	1	4
3	6	6	4	7	23
4	16	7	8	9	40
Total	26	16	18		

to the last two periods is shown in table 10. The calculated Chi-square showed

TABLE 10  
*Occurrence of unclean flavors between the first two and the last two periods  
for soybean and alfalfa hay*

Periods	Soybean hay	Alfalfa hay
1 and 2	7	12
3 and 4	35	28
Total	42	40

a highly significant difference in the occurrence of unclean flavors between the first two and the last two periods for soybean hay and a significant difference for alfalfa hay. Since the containers were cleaned thoroughly and sterilized, it is improbable that these unclean flavors came from the containers. At the start of the experiment, the hays seemed similar and of good quality. During period III, when large numbers of unclean flavors began to appear, the hays had a musty odor and appeared to have deteriorated during the experiment. Possibly the change in quality of the hays was responsible for the increased unclean flavors. Caution probably should be exercised when criticizing farmers in regard to the cleanness of equipment, because flavors which seem typical of unclean equipment possibly can be caused by poor quality hay.

Eighty-two samples of milk had unclean flavors. Of these, 26 occurred in the milk from the cows receiving ration A, 18 in the milk from those receiving

ration B, 16 in the milk from those receiving ration C and 22 in the milk from those receiving ration D. Chi-square was computed and the differences in occurrence of unclean flavors could not be attributed to any particular ration.

There seemed to be no influence of container on unclean flavor, for of 82 occurrences of this flavor, 25 occurred in glass, 25 in tin and 32 in rusty tin containers. These differences are not significant.

Rancid flavors occurred only 19 times in the 864 samples and could not be attributed to any particular ration. Every sample of milk was criticized as possessing a feed flavor. The intensity of the feed flavors ranged from slight to distinct. However, these flavors could not be attributed to any particular ration.

*Summary.* Two experiments were conducted to determine whether soybeans adversely affect the flavor of milk. The first was run during April to July; a grain ration containing cracked soybeans was compared with one containing linseed oil meal. Alfalfa hay was used as the only roughage. The soybean ration yielded milk with flavor scores equivalent to those of milk produced on a linseed meal ration. The type of container used for collection appeared to have no influence on milk flavors and scores during this season of the year.

The second experiment extended from November through May. Alfalfa hay and linseed oil meal were used as control feeds. Neither soybeans nor soybean hay adversely affected the flavor of the milk. A large number of unclean flavors appeared in the milk during the latter part of the experiment. The change in quality of the hay seemed to be responsible for the increase in unclean flavors that appeared in the milk. Contrary to the results of the first experiment, the rusty tin containers increased the susceptibility of milk to oxidation at this season of the year. The failure of the rusty containers to influence oxidative processes in milk during the first experiment may have resulted from the short time the milk samples were held in the rusty containers before scoring or to seasonal effects.

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# PARTURIENT PARESIS. IV. THE EFFECT OF UDDER INFLATION UPON BLOOD LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS IN COWS WITH PARTURIENT PARESIS<sup>1, 2</sup>

R. P. NIEDERMEIER AND VEARL R. SMITH

*Department of Dairy Husbandry, University of Wisconsin*

Air inflation of the udder was used extensively as a treatment for parturient paresis until the development of calcium therapy. Even today, air inflation is resorted to in cases that do not respond to calcium therapy (23). Seitter (25) reported that inflation of the udders of anesthetized goats and cows produced a marked increase in blood pressure. Later Auger (4), using a more refined technique, concluded that air inflation caused only a slight increase in blood pressure. Maguire (20), Widmark and Carlens (28) and Auger (3) report a marked increase of blood sugar after inflation of the udder with air. Fish (10, 11), Sjollem (26) and Hayden (17) demonstrated that the hyperglycemia occurring after udder inflation resulted from lactose in the blood, presumably absorbed from the mammary gland.

Several workers have shown an increase in blood serum calcium following mammary inflation for milk fever treatment (9, 16, 27). Fish (12) demonstrated an increase in both serum calcium and inorganic phosphates. The mechanism whereby udder inflation produces the above effects has been a source of much conjecture. Peterson and Rigor (19) and Garrison and Turner (12) have reported that milk secretion is practically inhibited when air pressures of 25 to 40 mm. Hg are maintained in the cow's udder. Some (9, 19) attribute the effect of inflation to the cessation of milk secretion, thereby preventing the further uptake of milk precursors from the blood. Others (6, 15) postulate that udder inflation gives rise to afferent stimuli which are responsible for the curative effects.

This work was undertaken to obtain, in more detail, the changes in calcium, magnesium and inorganic phosphorus during recovery from parturient paresis after udder inflation. At the time the work was initiated, no one had reported studies on magnesium levels after inflation. In a recent study of parturient paresis treated by air inflation, Robertson (23, 24) included data on magnesium levels.

## EXPERIMENTAL PROCEDURE

The udders of seven Jersey cows with parturient paresis were inflated with air to a pressure of 60 to 70 mm. Hg and the teats taped to prevent the escape of air. The pressure was measured with an aneroid manometer attached to a teat

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<sup>1</sup> Part of these data were taken from a thesis presented by R. P. Niedermeier to the graduate faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup> Published with approval of the Director of the Wisconsin Agricultural Experiment Station.

TABLE 1  
The effect of udder inflation on calcium, phosphorus and magnesium of cows with parturient paresis

Cow no.	12			20			63			494			556			705			732					
Time	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg			
(hr.)	(mg. %)																							
	Precipitation						Postinflation																	
	4.6	1.3	2.0	5.2	0.5	3.3	4.6	1.4	4.5	2.8	1.2	2.6	4.4	0.8	2.8	3.1	0.62	3.5	4.5	1.4	4.1			
0.5	4.8	2.1	1.9	5.0	0.5	3.1	5.1	1.5	4.6	3.4	2.1	2.8	5.1	0.7	2.8	3.7	0.87	3.7	5.1	2.2	3.9			
1.5	4.7	1.5	2.2	5.1	0.7	3.3	6.1	2.3	4.6	3.8	3.0	2.6	5.4	0.9	2.8	4.3	1.09	3.8	5.6	2.7	4.0			
3.0	5.4	1.7	2.2	5.1	0.7	3.5	6.8 <sup>a</sup>	2.5	4.7	4.0	2.2	2.6	4 <sup>a</sup>	1.0	3.0	4.1	1.06	3.7	5.4	2.9	3.7			
5.0	5.9	2.7	2.3	6.0	1.0	3.4	6.3	2.6	4.5	5.3	2.5	2.8	4.9	0.8	3.1	4.3	1.23	3.7	6.0 <sup>a</sup>	2.9	3.6			
8.0	6.0	3.3	2.4	6.9 <sup>a</sup>	1.2	3.5	6.6	2.9	4.3	5.7 <sup>a</sup>	3.3	3.1	5.0	1.4	3.2	3.3	1.15	3.7	6.8	2.7	3.4			
11.0	6.7	3.8	2.7	7.3	1.4	3.5	6.5	3.0	4.3	5.2	3.6	3.2	5.4	3.7	3.5	4.8	0.87	3.6	7.5	2.1	3.6			
14.0	7.1 <sup>a</sup>	4.7	2.4	7.6	1.8	3.5	6.8	3.0	4.3	5.1	2.8	2.9	6.0	3.5	3.2	4.6	1.62	3.8	7.4	3.3	3.5			
17.0	8.2	6.0	2.6	7.7	1.9	3.0	6.9	2.1	4.3	5.0	1.6	2.8	6.1	3.2	3.3	4.6	2.13	3.8	7.3	4.1	3.3			
20.0	8.5	6.4	2.6	7.6	2.8	3.2	7.1	3.3	3.9	5.0	1.8	2.9	6.8	2.8	3.6	4.0	1.70	3.9	7.5	4.4	2.9			
36.0							6.1	6.0	3.5										9.8	3.6	2.3			
48.0							8.6	6.3	2.6										9.8	3.8	2.0			

<sup>a</sup> Time after inflation when cow was found standing.



cannula. The cows were not treated until they were down and unable to rise. A sample of venous blood was drawn before inflation. Post-inflation samples were drawn at 0.5, 1.5, 3, 5, 8, 11, 14, 17, 20, 36 and 48 hr. Samples were analyzed for serum calcium, serum magnesium and inorganic phosphorus, and all analyses were made in duplicate. Blood serum calcium was determined by the method of Clark and Collip (7), serum magnesium according to Simonsen *et al.* (26) and plasma inorganic phosphorus by the Fiske and Subbarow method (13).

#### RESULTS AND DISCUSSION

Table 1 shows the results of analyses of blood samples for total serum calcium, serum magnesium and plasma inorganic phosphorus. Cow 494 was in a coma at the time of inflation. Cows 20 and 494 were reinflated 3 hr. after the first inflation as a precautionary measure, but no relapses occurred. The time relationships for calving, occurrence of milk fever, treatment and recovery are shown in table 2. Recovery was regarded as the time the cow arose to her feet and stood up of

TABLE 2  
*Summary of time of treatment and recovery in relation to calving*

Cow no.	Parturient paresis		Inflation completed		Recovery	
	Time <sup>a</sup>		Time <sup>a</sup>		Time <sup>a</sup>	
	Hr.	Min.	Hr.	Min.	Hr.	Min.
12	6	00	7	40	9	40
20	7	40	9	00	17	00
63	39	00	46	10	49	10
494	17	40	19	55	27	55
556	15	30	17	00	20	00
705 <sup>b</sup>	11	45	14	30	19	30
732	22	00	28	10	33	10

<sup>a</sup> Measured from end of calving.

<sup>b</sup> Suffered relapse 38 hr. after calving and was treated with an intravenous injection of calcium gluconate. A second injection of calcium gluconate was made 76 hr. after calving and the cow died suddenly, after apparent recovery 9 hr. after the second injection.

her own accord. Time of recovery ranged from 3 to 14 hr. after treatment by udder inflation.

The severity of the milk fever cases is reflected in the low total serum calcium and phosphorus in all pre-inflation samples. Inorganic phosphorus values as low as 0.5 mg. per cent also were reported by Allcroft (2) for milk fever cows. With the exception of cows 556 and 705, recovery was noted when the calcium level was near or above 6 mg. per cent. Cow 494 remained on her feet with a serum calcium level of only 5 mg. per cent at 20 hr. after inflation. In the seven cases studied, plasma phosphorus levels increased following udder inflation, but in no case could the phosphorus level be considered within the normal range when the cows got up. Blood serum magnesium levels remained in the normal to high normal range as reported by Allcroft (2) in all cows except 63 and 732, where a hypermagnesemia condition existed. Recovery was uneventful in all cows except 705. In 705 the calcium and phosphorus did not return to normal up to 20 hr. after inflation. Shortly after 20 hr. post-inflation, she had a relapse and

was treated with calcium gluconate. Two days after the first attack 705 died suddenly after apparent recovery.

Robertson (23) found no correlation upon statistical analysis of his data on 19 milk fever cases between blood magnesium levels and the symptomatology classification as proposed by Barker (5). Pribyl (22) suggested the symptoms of milk fever may be due to a magnesium narcosis. Hibbs *et al.* (18) reported that upon intravenous injection of magnesium sulphate into cows, general anesthesia resulted when the serum magnesium reached a level of 7.5 mg. per cent. When the serum calcium then was raised to 17.5 mg. per cent by an injection of calcium gluconate, the cows regained consciousness, even though the serum magnesium remained at the same high level. They suggested that the relationship between serum calcium and serum magnesium may be of great importance in the symptomatology of milk fever. Allcroft (1) found that anesthesia was produced in goats when the serum magnesium was between 13.5 and 14.5 mg. per cent, with the serum calcium around 7 mg. per cent. He further comments (2) on the importance of the calcium and magnesium ratio, as well as the calcium phosphorus ratio in milk fever.

These data show that there is a considerable difference between cows as to the blood levels of calcium, phosphorus and magnesium at the time of recovery. This suggests that possibly the relative levels of the calcium, magnesium and phosphorus are more important in the symptomatology of milk fever than the actual blood level of any one constituent.

#### SUMMARY

Data are given for the blood levels of calcium, magnesium and phosphorus during the recovery period for seven cases of parturient paresis in Jersey cows which were treated by udder inflation.

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# FERTILITY AND LIVABILITY OF BULL SEMEN DILUTED AT VARIOUS LEVELS TO 1:300

E. L. WILLETT

*American Foundation for the Study of Genetics, Madison, Wisconsin*

The phenomenal growth of the practice of artificial insemination of dairy cattle in the United States has necessitated the development of means of increasing the number of cows that can be bred to a given sire. This problem is of extreme importance because of the limited number of sires proved to be transmitters of satisfactory levels of milk and fat production. One of the most promising and fruitful solutions has been that of semen dilution.

A series of experiments concerning the fertility of semen diluted at various levels has been conducted by Salisbury and associates. In the first three papers (6, 8, 11) increasingly high dilutions were tried until it was demonstrated that levels as high as 1:100 could be used without lowering breeding efficiency. These studies have been of inestimable value to the dairy industry in that they, along with the development of the egg yolk diluter (5), have made possible the great expansion of the practice of artificial insemination. In later experiments (10) it was shown that, with increase in dilution levels above 1:100, there was a progressive decline in breeding efficiency.

The American Foundation for the Study of Genetics also has been conducting research along this line. During the past 2 yr. a series of controlled experiments has been conducted to determine the fertility of semen diluted above 1:100. It is believed that enough information has been accumulated to enable the establishment of quantitative results. The information obtained from these studies is presented below.

## EXPERIMENTAL PROCEDURE

Four experiments were conducted in which each semen collection was split three ways. One-third was diluted 1:100, and the other two portions were diluted at higher levels. Each collection consisted of two or more ejaculates which were mixed together before being added to the diluters. The different dilution levels were rotated among different inseminator groups where the semen was used for breeding. As far as could be predicted when planning each experiment, these groups were equal in regard to number of cows bred and in breeding efficiency. Each experiment, therefore, consisted of two or more Latin squares. In experiments 2 and 4 each Latin square consisted of three collections from a given bull. The semen was from Guernsey and Holstein bulls selected for high fertility when used artificially.

The non-returns in the first three experiments were determined 60 to 90 days after service and were based on first and second services made the day following

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collection. In the fourth trial the non-returns were obtained in a similar manner except that, in an attempt to reduce variation, they were determined 72 to 78 days after service. Figures computed in this way are comparable to the 60- to 90-day values because in both cases 75 days is the average or midpoint.

In experiments 1 and 3 spermatozoan concentration was determined by opacity, using the method of Salisbury *et al.* (9). They observed a correlation coefficient of 0.98 between spermatozoan numbers and opacity. A study has been made by Willett (13) of 110 separate ejaculates from 29 bulls wherein the number of spermatozoa were determined both by the above-mentioned method and by a haemocytometer. With the latter, the semen was diluted 1:100 with 0.85 per cent NaCl solution containing a small quantity of chlorazene to kill the spermatozoa. In each case, the cells in all the squares in a ruled area were counted. A correlation coefficient of 0.786 between spermatozoan numbers and 2-(logarithm of light transmission) was obtained when comparing these two methods. Only 62 per cent of the variation in light transmission was, therefore, accounted for by number of spermatozoa. These unsatisfactory results probably were due to the presence of interfering substances in the semen. Perhaps this difficulty would be overcome by adaptation of the technique devised by Emik and Sidwell (4) for ram semen. This technique was published after most of the dilution experiments reported in this paper were completed. In experiments 2 and 4, which were larger in scope than the others, the most accurate spermatozoan counts possible were desired. As a consequence, haemocytometer counts were made as described above.

Experiments 1 and 3 were preliminary in nature and small in scope. In the first, nine collections from five bulls were used, while in the third there were six collections from six bulls. The two large-scale trials, numbers 2 and 4, consisted of 18 collections from 6 bulls and 36 collections from 12 bulls, respectively.

The yolk-citrate diluter (36.0 g.  $\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  per liter of water in the buffer) was used in the first experiment. In the later ones the same diluter with 6 g. sulfanilamide per liter of buffer was used.

## RESULTS

*Fertility of semen.* The breeding results from the four experiments, along with average figures for spermatozoan numbers, are presented in table 1. The

TABLE 1  
Average non-returns from, and number of spermatozoa in, semen diluted at different levels in four experiments

Expt. no.	No. of services	Av. percentage non-returns and sperm numbers by dilution levels				
		1:100	1:125	1:150	1:200	1:300
1	2,146	60.7(12.3) <sup>a</sup>	57.5(9.8)	57.0(8.2)		
2	3,449	68.3(12.7)		68.3(8.5)	65.6(6.4)	
3	1,441	63.9(13.0)			63.6(6.6)	59.9(4.4)
4	4,338	65.0(10.1)			61.9(5.1)	59.5(3.4)

<sup>a</sup> Figures in parentheses are av. no. (in millions) of spermatozoa per ml. of diluted

number of services for each dilution level is not given, as the figures for each experiment are approximately the same and, for all practical purposes, may be considered equal. It can be seen that in every experiment there is a downward trend with increase in dilution rate or with decrease in number of spermatozoa. None of these differences is significant. Experiments 2 and 4 were designed to enable the statistical measurement of bull and dilution interaction. In neither experiment is this interaction significant, however. If bulls selected at random from the population and not those selected for high fertility had been used in the experiment, it seems logical that a significant interaction would have been obtained. This laboratory has additional limited data suggesting that reduction in breeding efficiency greater than that observed above can result when semen from a bull of questionable fertility is diluted above 1:80.

Salisbury and Bratton (10) reported two experiments wherein citrate-yolk diluter was used in one and citrate-sulfanilamide-yolk was used in the other. With the latter diluter they observed less reduction in non-returns with increase in degree of dilution than with the citrate-yolk. The data presented in table 1 are in line with this observation, for sulfanilamide was used only in the last three experiments. Direct comparisons within one experiment, however, are needed to demonstrate definitely if such differences actually occur.

In the course of the analyses of the data in experiments 2 and 4, the non-return percentages were plotted on graphs against numbers of spermatozoa per ml. of diluted semen. Over-all regression coefficients were calculated. The relationship on the graphs tended to be curvilinear, with the slopes being greater when spermatozoan numbers were less than 6 million. The data from each experiment were, therefore, divided into two groups, and separate regression coefficients were calculated. One group contained samples with 6 million or more spermatozoa per ml. and the other less than 6 million. The over-all range in spermatozoan numbers in each of the two experiments were 4.6 to 17.8 and 1.5 to 17.2 million, respectively. The regression coefficients are presented in table 2. Al-

TABLE 2

*Regression of non-return percentages on spermatozoan numbers by individual samples*

Expt. No.	All samples		Samples with 6 million or more spermatozoa/ml.		Samples with less than 6 million spermatozoa/ml.	
	No.	b <sup>a</sup>	No.	b	No.	b
2	54	0.77**	47	0.43	7	6.99
4	108	0.78*	44	0.52	61	2.62*

<sup>a</sup> b = regression coefficient—the drop in non-return percentages per million decrease in number of spermatozoa in each ml. of diluted semen.

\* Significant. Probability = 0.05 or less

\*\* Highly significant. Probability < 0.01.

though they suggest curvilinearity, the coefficients representing the two groups in each experiment are not significantly different. More data are needed to establish definitely this relationship.

The two over-all coefficients, 0.77 and 0.78, agree very closely with the corre-

sponding figure of 0.8 given by Salisbury and Bratton (10) for their large-scale experiment. The figures of 0.43 and 0.52 are somewhat higher, however, than that of 0.3 calculated by them from 700 ejaculates used routinely for breeding and with a range of 6,700,000 to 34,600,000 spermatozoa per ml. Apparently these samples included many which were diluted at levels less than 1:100. When such samples are included, the regression coefficient probably is lower than if they were not used. They mentioned that the data from their controlled experiments were not curvilinear even though their ranges in spermatozoan numbers were comparable to those reported in this paper.

As can be seen from the data in table 1, when semen is diluted at 1:100 it contains on the average about 12 million spermatozoa per ml., and at 1:200, 6 million. On the basis of the data presented above, therefore, there is a decline of about 3 per cent in non-return rate when the dilution level is extended from 1:100 to 1:200. This figure determined by means of the regression coefficients agrees closely with the information obtained directly by comparing in table 1 the non-returns for these two dilution levels.

In Salisbury and Bratton's (10) experiment using citrate-sulfanilamide-yolk diluter and in the experiments reported in this paper, it has not been possible to demonstrate significant differences in non-returns between dilution levels of 1:100 and 1:200. Their difference is 2.5 per cent. Apparently the experiments have not been sensitive enough or on a large enough scale to establish significance. The differences have been quite consistent, however, and, therefore, probably can be assumed not to be due to chance.

It must be kept in mind that, in the four experiments reported in this paper, the data are based on inseminations made the day following collection of semen. The maximum storage time was approximately 36 hr. Thus the results may not be applicable to longer storage periods. Non-return rates calculated from the few services made the second day following collection indicate that there was an over-all downward trend in non-return rate with increase in age of semen but that the relative difference between the dilution levels remained about the same.

By the extension of dilution levels from 1:100 to 1:200 the number of cows that could be bred to a given bull would be almost doubled. It seems reasonable that a sacrifice of 3 per cent in non-return percentage could sometimes be made in order to utilize outstanding sires to the maximum, especially if breeding efficiency is otherwise at a high level. The question of whether or not to make such a sacrifice is one of economics and must be decided by the stud manager in accordance with his individual situation.

*Livability of spermatozoa.* While the four experiments were being conducted, observations were made of spermatozoan motility in all samples at 2-day intervals until the semen had been stored in a refrigerator at 4° C. for 12 days. Simultaneously with the fourth experiment another study which included duration of motility at the dilution level of 1:50, was being made of the semen from most of the collections. Since motility observations might be of interest because of the wide range in dilutions, the data from the collections studied in both experiments were combined and are presented in table 3. The semen was graded

TABLE 3

*Average motility (in per cent) during storage of spermatozoa in semen diluted at four levels, and number of samples containing no motile spermatozoa at 12 days.*  
*(Observations per datum: 32. Total observations: 896.)*

Time	Av. % motility at dilutions of:				L.S.D. <sup>a</sup>
	1: 50	1: 100	1: 200	1: 300	
Fresh	73	73	73	73	
2 d.	68	65	59	56	3
4 d.	59	57	48	44	5
6 d.	51	43	35	29	5
8 d.	43	34	24	18	5
10 d.	33	21	13	12	4
12 d.	23	12	8	4	4
Samples with no motile spermatozoa at 12 d.	0	6	11	15	

<sup>a</sup> Least difference required for probability of 0.01.

on the basis of the per cent of spermatozoa showing any degree of motility. Estimates were made to the nearest 10 per cent. In the table, for each storage period the least significant difference required for a probability of 1 per cent is given. The numbers of samples containing no motile spermatozoa on the twelfth day of storage also are given because the determination of the presence or absence of motile spermatozoa is considered to be more objective than determination of motility percentages. By means of the chi-square test it was determined that the probability, according to Crow's chi-square chart (3), of the differences in number of dead samples being due to chance is 0.0001. Whichever criterion is used, it can be seen that the livability of spermatozoa during storage decreases with increase in dilution rate. These livability studies are in line with the fertility studies reported above. Salisbury *et al.* (8, 12) observed a similar decline in livability with increase in rate of dilution with dilution levels up to 1:100.

These livability studies indicate that there is much room for improvement of the diluters now available. Salisbury (7) attributed the effect of high dilutions to the harmful action of oxygen. In later work, Van Demark *et al.* (12) presented data which indicate that oxygenation is mainly but not solely responsible for lowered livability of spermatozoa stored for a number of days in semen diluted at high levels. Studying the immediate effect of dilution upon motility, Cheng *et al.* (2), on the other hand, present evidence indicating that oxygen is not a factor and suggest that the "leaching" of one or more necessary substances from the spermatozoan may be the contributing cause of the harmful effect of high dilutions. This problem merits additional study.

*Correlation studies.* The data from experiment 4 were used to determine the correlation between non-return rates, spermatozoan numbers and motility of spermatozoa after either 2 or 8 days of storage. Calculations were made with all samples together, with samples containing 6 million or more spermatozoa per ml. and with samples containing less than 6 million spermatozoa.



The coefficients significant only at the 5 per cent level of probability were for non-returns and spermatozoan numbers with all samples (0.23) and with samples with less than 6 million spermatozoa (0.24), and also for non-returns and spermatozoan numbers with 2-day motility held constant by partial correlation using all samples (0.23). The only correlation significant at the 1 per cent level of probability was between sperm numbers and 8-day motility using all samples (0.29).

The correlation analyses indicate that decrease in spermatozoan numbers was a more important factor causing reduction in breeding efficiency with increase in dilution rates than the depression of spermatozoan motility by the high dilutions. The correlations between spermatozoan numbers and nonreturn rates were changed only slightly when the effect of motility was held constant.

These results are somewhat contrary to those obtained by Cheng and Casida (1) with rabbits in a study to determine the number of spermatozoa required for maximum and partial fertility. Their correlation coefficients indicated that the effect of dilution upon motility was of much greater importance than the actual reduction in numbers of spermatozoa. In their work a much wider range in dilution levels was used than in the experiments reported in this paper. With comparable dilution levels comparable results might have been obtained. Cheng *et al.* (2) have studied the effect of high dilutions upon motility of unstored bull spermatozoa in yolk-citrate. Their data suggest that the immediate effect upon motility of the dilution levels compared in experiment 4 would be slight.

The significant correlation of 0.23 between non-returns and sperm numbers obtained in experiment 4 with all samples is almost identical to that of 0.24 obtained by Salisbury and Bratton (10).

#### SUMMARY AND CONCLUSIONS

1. Four controlled experiments were conducted to study dilution levels above 1:100. A total of 11,372 services from 69 collections from bulls selected for high fertility were involved. In every experiment there was a downward trend with increase in dilution level, but none of the differences was significant.

2. In two of these experiments with a total of 7,787 services from 54 collections, accurate spermatozoan counts were made and regression coefficients were calculated. The citrate-sulfanilamide-yolk diluter was used. The relationship between non-return percentages and spermatozoan numbers at dilutions above 1:100 appears to be curvilinear.

3. As the number of spermatozoa decreased from approximately 12 million to 6 million, there was a decrease of approximately 0.5 in non-return percentage per million decrease in number of spermatozoa per insemination. Within this range in spermatozoan numbers, which corresponds roughly to dilution levels of 1:100 and 1:200, respectively, when 1 ml. of semen is used per insemination, there is, therefore, an over-all decline in non-returns of approximately 3 per cent.

4. When the diluted semen contained less than 6 million spermatozoa per ml., there was a drop of over 2.6 per cent in non-returns per million decrease in number of spermatozoa per insemination.

5. Motility observations are reported on a total of 128 samples from 32 collections studied at 2-day intervals during a storage period of 12 days. Dilution levels of one to 50, 100, 200 and 300 were compared. There was a marked decrease in livability of spermatozoa with increase in dilution rate.

6. Correlation analyses indicate that decrease in spermatozoan numbers was more important in causing the decrease in non-return rates with the dilution levels studied in this experiment than the direct depression of motility by high dilutions.

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## STALE-FLAVOR COMPONENTS IN DRIED WHOLE MILK. II. THE EXTRACTION OF STALE BUTTER OIL FROM STALE DRIED WHOLE MILK BY ORGANIC SOLVENTS

R. MCL. WHITNEY AND P. H. TRACY

*Department of Food Technology, University of Illinois, Urbana*

In a previous paper (4), it was reported that the stale-flavor components which develop in spray-dried whole milk during storage were found to be concentrated in the butter oil when prepared according to the usual method. However, due to unavoidable homogenization in the spray-drying process, the recovery of butter oil from the dried whole milk, and hence the removal of the stale-flavor components was very inefficient (approximately 35 per cent). Consequently, the possibility of obtaining stale butter oil directly from the whole milk powder by extraction with organic solvents was investigated.

For such a method to be applicable, the recovery of the butter oil must be reasonably high, the stale-flavor components must be extracted with the butter oil and the solvent must not interfere in any way with the flavor judgments of the products.

### THE EFFICIENCY OF THE EXTRACTION PROCEDURE

Preliminary work indicated that the stale-flavor component could be extracted with the butter oil from stale, spray-dried whole milk by organic solvents. However, suitable techniques would be needed to improve the efficiency of the extraction procedure and to prevent the interference of solvent flavors with the judgment of the product. In order to improve the efficiency of extraction of the butter oil from the spray-dried whole milk, several modifications involving the type of whole milk powder extracted, the pretreatment of the powder before extraction and the extracting solvents were investigated.

*Manufacture and storage of dried whole milk.* Two types of dried whole milk were prepared in a pilot-size experimental spray drier. Powder no. 104 was manufactured from condensed whole milk without previous homogenization, while powder no. 105a was made from uncondensed, unhomogenized milk. The conditions of manufacture and storage are indicated in table 1.

*General experimental methods.* In all experiments, a weighed sample of the milk powder of known fat content, as measured by the Mojonnier method, was pretreated in the manner specified and extracted in a Soxhlet apparatus with a measured volume of solvent. The solvent was maintained at the boiling point by immersing the flask in a constant-temperature water bath. After the specified number of trips of the syphon, the solution was removed from the Soxhlet apparatus and the solvent evaporated from the butter oil under reduced pressure. The extracted butter oil was weighed to determine the efficiency of the method.

The anhydrous ethyl ether used was prepared by redistilling U.S.P. grade

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ethyl ether over metallic sodium. The petroleum ether was distilled over solid KOH and the fraction boiling between 30 and 41° C. collected.

*Experimental results.* While a considerable number of extractions were performed, only typical examples of the various procedures are reported in table 2. In experiments 1 and 2, powder no. 104, was extracted with anhydrous ethyl ether and with petroleum ether, respectively, without pretreatment. Poor recoveries were obtained with both solvents.

TABLE 1  
*Manufacturing data for dried whole milk*

	Batch no.	
	104	105a
Raw milk:		
Source	University herd	University herd
Fat content (%)	4.3	3.9
Preheat treatment:		
Temp. (° C.)	77-79	71
Time (min.)	21	30
Condensing:		
Temp. (° C.)	46-71	none
Vacuum (mm. of Hg)	49-64	
Time (hr.)	1.2	
Total solids (%)	35.24	
Fat content (%)	11.37	
Cooling & storage:		
Final temp. (° C.)	12-15	
Method	surface cooler	
Storage time (hr.)	22.5	
Storage temp. (° C.)	~ 7	
Drying:		
Spraying temp. (° C.)	54-61	55-61
Spraying pressure (lb./in. <sup>2</sup> )	450-550	450-600
Spray nozzle size	63-17	69-20
Inlet temp. (° C.)	135-154	121-149
Outlet temp. (° C.)	85-97	82-107
Fat content (%)	31.60	29.56
Moisture content (%)	2.07	2.33
Solubility index	0.6	< 0.1
Powder storage:		
Time of storage (mo.)	> 8	< 1
Temp. of storage (° C.)	~ 7	~ 7

Since Holm *et al.* (2) had reported that the fat is more readily extracted by carbon tetrachloride from a powder prepared from uncondensed, unhomogenized milk, powder no. 105a was extracted without pretreatment in experiments 3 and 4. Satisfactory recoveries were obtained with both solvents. However, this powder developed a tallowy flavor quickly, which made it unsatisfactory for the investigation of the stale flavor.

Lampitt and Bushill (3) had observed that the amount of fat that could be extracted by organic solvents from dried whole milk prepared by the usual spray-drying process was increased by hydrating the powder to approximately 8 per

cent moisture. In experiment 5, dried whole milk no. 104 was stored in evaporating dishes and placed in desiccators over water at room temperature. After storage for 44.25 hr. under these conditions, the moisture content of the powder, as determined by the toluene-distillation method, was 7.87 per cent. Extraction of this powder with anhydrous ethyl ether yielded satisfactory recoveries. However, the time required to hydrate the powder by this method was longer than desirable.

The dynamic method suggested by Wilson (5) was employed in experiment 6. Air was drawn by means of a high-vacuum pump through the following succes-

TABLE 2

*Typical results of various methods for the solvent extraction of butter oil from dried whole milk*

	Expt. no.						
	1	2	3	4	5	6	7
Powder no.	104	104	105a	105a	104	104	104
Moisture content (%)	2.07	2.07	2.33	2.33	2.07	2.07	2.07
Special pretreatment	none	none	none	none	static hydration	dynamic hydration	alcohol treatment
Moisture content after pretreatment (%)					7.87	9.20	
Wt. of powder (g.)	40.00	40.03	40.00	40.00	176.8	40.01	402.1
Fat content of powder (%)	31.60	31.60	29.56	29.56	29.73	29.30	31.60
Solvent	anhydrous ethyl ether	petroleum ether b. p. < 41° C.	anhydrous ethyl ether	petroleum ether b. p. < 41° C.	anhydrous ethyl ether	petroleum ether b. p. < 41° C.	petroleum ether b. p. < 41° C.
Vol. of solvent (ml.)	400	300	400	500	750	340	2620
Temp. of extraction (°C.)	43	49	43	49	40	43-49	47-48
Time of extraction (hr.)	3.5	6	3.25	3	4.25	5.5	4.5 <sup>a</sup> 11.0 4.25
No. of trips of syphon		18			19	19	18 <sup>a</sup> 19
Wt. of extracted butter oil (g.)	1.00	4.31	11.50	9.30	51.2	11.11	20 115.5
Recovery of butter oil (%)	7.9	34.1	97.5	78.5	97.5	94.6	90.9

<sup>a</sup> The powder was divided equally between extraction thimbles. The variations in time of extraction are due to different periods for tripping of the syphon caused by differences in construction of the extraction apparatus used.

sive stages: a charcoal adsorption tube, a washing bottle filled with water maintained at room temperature by immersion in a water bath, a modified Regnault dew point hygrometer, a tube containing 600 g. of dried whole milk no. 104 and a second hygrometer. At 1-hr. intervals, the absolute humidity of the air on both the inlet and outlet sides of the powder was determined. Throughout the hydration period, the air in the inlet side was maintained at as near saturation as possible by regulating the flow of air with a stopcock at the pump. The absolute

humidity was 22.8 to 24.3 mm. of mercury on the inlet side and 2.4 to 9.5 mm. of mercury on the outlet side, while the air temperature was 24.0 to 25.8° C. After 25 hr. of treatment, the moisture content of the dried whole milk was determined by the toluene-distillation method and a weighed sample extracted with petroleum ether in the usual manner. Satisfactory recoveries of butter oil were obtained.

To further reduce the time required for pretreatment of the dried whole milk, the possible application of an observation of Lampitt and Bushill (3) was studied. These investigators had noticed that, in freeing the fat for extraction by hydration of the milk powder to a moisture content of approximately 8 per cent, the lactose was crystallized. They also found that the lactose crystallized when the powder was dispersed in 95 per cent ethyl alcohol. To test whether pretreatment of the powder with alcohol also would free the fat for extraction and to develop the most satisfactory technique, a variety of experiments were performed. Experiment 7 illustrates the most satisfactory procedure used. Approximately 400 g. of dried whole milk no. 104 was agitated with 3,400 ml. of 95 per cent ethyl alcohol and 25.8 ml. of distilled water for 1 hr. and filtered. The alcoholic filtrate was divided into three portions, each of which was concentrated to approximately 850 ml. by evaporation under vacuum at room temperature. Eight hundred and fifty ml. each of petroleum ether and distilled water then were added to each portion and the mixture agitated for 2 min. in a separatory funnel. After the alcohol-water layer had been discarded, the petroleum-ether layer was used to extract the treated powder. Satisfactory recoveries of fat were obtained.

*Discussion.* In interpreting the results of this study, it must be kept in mind that the purpose was not to explore completely the effect of various factors upon the extraction procedures, but rather to develop efficient and usable methods for this step in the isolation of the stale flavor component. Therefore, no conclusions are justified with respect to small differences in recovery of butter oil, but certain general observations are possible.

While there were several differences in the methods of manufacture and storage of the two dried whole milks extracted, the chief difference was the drying of a condensed (powder no. 104) and an uncondensed (powder no. 105a) milk. The results of the first four experiments, may be considered as confirming the observation of Holm *et al.* (2) that fat is extracted more completely by organic solvents from a powder prepared from an uncondensed than from a condensed product. Unfortunately, the powder prepared from the uncondensed whole milk was so unstable that it was unsuitable for the study of stale flavor. A reasonable hypothesis for the instability of this product is that the physical condition of the powder which makes the fat more available for solvent extraction also makes it more susceptible to oxidation.

The results of the experiments in which the dried whole milk was hydrated to approximately 8 per cent moisture before extraction confirm the observations of Lampitt and Bushill (3) that the fat was readily extracted by organic solvents from dried whole milk when pretreated in this manner. As was expected, the rate of hydration was more rapid when the dynamic method of Wilson (5) was used.

The experiments employing the alcohol pretreatment indicate that satisfactory recoveries of butter oil can be obtained by agitation for 1 hr. of the dried whole milk with 95 per cent ethyl alcohol to which sufficient water has been added to hydrate the powder to 8 per cent moisture followed by extraction with petroleum ether in the usual manner, provided the fat dissolved in the alcohol used in the pretreatment is recovered. The reaction involved in the freeing of the fat for extraction by the alcohol pretreatment apparently approaches completion in 1 hr. However, shorter periods of treatment were not investigated. Some slight improvement in recovery was indicated by experiments in this series when water was added to the 95% alcohol. However, additional information is needed before any conclusions concerning the optimum amount to be used can be made. Several hypotheses may be suggested for the mechanism of the process whereby the fat is freed by the alcohol pretreatment. The alcohol may serve as a medium by which the water may be brought into more intimate and continuous contact with the lactose in the milk powder, thus speeding the crystallization process. This, in turn, would free the fat for extraction by destroying the continuity of the lactose glass which may entrap the fat. In this case there should exist between the alcohol and the lactose a competition for the water and additional water in the alcohol should shift the equilibrium toward the formation of more crystalline lactose and the consequent freeing of more fat for extraction. However, the evidence is only suggestive and the other possibilities that the alcohol simply serves as a medium in which the lactose crystals may grow or that the alcohol may effect the other components of the powder so as to make it more porous cannot be eliminated.

#### A METHOD FOR THE REMOVAL OF SOLVENT FLAVORS FROM BUTTER OIL

When the solvents were removed from the butter oils in the previous study by evaporation under vacuum at approximately 40° C., the butter oil, reconstituted with fresh skim milk to the approximate composition of the original milk (3.8 per cent fat), possessed sufficient solvent flavor to interfere with the establishment of its stale-flavor threshold value. Investigation of other common methods of solvent removal did not yield satisfactory results. Hence, a method specifically designed for this purpose was developed.

*Experimental Method.* Briggs (1) had observed that, by the adsorption of gases on activated cocoanut charcoal at liquid-air temperatures from low-pressure systems, higher vacuums could be obtained than by use of high-vacuum pumps alone. Also it is known that organic solvents are adsorbed strongly by charcoal at low temperatures. Therefore, the special tube shown in fig. 1 was constructed. Butter oil and the solvent to be investigated were added to the pyrex flask A. Cocoanut charcoal (~31 g.) was placed in the pyrex tube B, which was connected to the flask by means of a ground-glass joint. The flask A and its contents were held at a constant temperature of 40° C. in water bath, while the entire apparatus was agitated continuously by a Boerner shaker. The bulk of the solvent was removed with an aspirator through stopcock C, and then a vacuum pump was attached and the pressure reduced to 0.1 mm. of mercury or less. After the sys-

tem had been evacuated for a sufficient length of time, the charcoal in the tube B was degassed by being heated with a Bunsen burner while the pump was still in operation. The heating period (usually 15–20 min.) was complete when no change in the pressure of the system could be noted after the charcoal had been heated for a 5-min. period in a closed system. With the stopcock still closed, the charcoal bulb was allowed to cool to room temperature, and then the pressure of the system was further reduced to  $10^{-5}$  mm. of mercury or less by immersion of the bulb in liquid air contained in a 1 l. Dewar flask. After a sufficient time, the bulb was removed from the liquid air, the stopcock C was opened and the

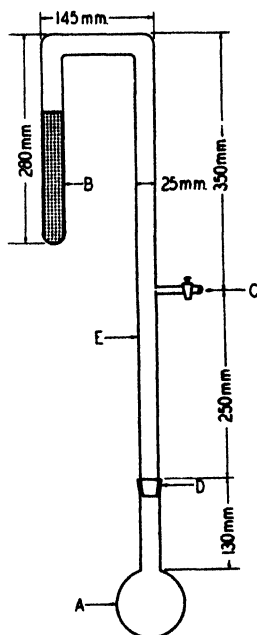


FIG. 1. Apparatus for removal of solvents from butter oil: A—500 ml. pyrex flask for butter oil and ether. B—Cocoonut charcoal (31 g.). C—Stopcock leading to vacuum pump. D—Ground glass fitting. E—25 mm. pyrex tube.

flask containing the butter oil was disconnected from the bulb. The pressure in the system during the process was determined by means of a high-vacuum arc tester which gives a pale-blue arc at less than 0.1 mm. of mercury pressure and no discharge at  $10^{-5}$  mm. of mercury pressure or less. While this method for measuring the pressure may seem somewhat qualitative, it was satisfactory for the purpose of this study.

*Time study.* To determine the minimum time required for evacuation with the high-vacuum pump and for the charcoal adsorption for the solvents used in this study, the duration of these treatments was varied and the butter oils obtained, blended with fresh skim milk to 3.8 per cent fat and scored for solvent flavor by



a panel of experienced judges. Their observations are recorded in table 3.

*Discussion.* In the development of this procedure no attempt was made to determine the effect of changes in such variables as weight and type of charcoal, design of apparatus, or amount of butter oil, since a satisfactory method was de-

TABLE 3

*Effect of time of evacuation and time of charcoal adsorption upon the completeness of solvent removal from butter oil*

Sample no.	Solvent	Wt. of butter oil	Vol. of solvent	Time of evacuation	Time of adsorption on charcoal	Judging		
						No. of judges	Controls	Treated samples <sup>a</sup>
		(g.)	(ml.)	(hr.)	(hr.)			
1	ethyl ether	40	400	21.8		4	2 + 2 -	4 +
2	ethyl ether	40	400	21.8	1.0	4	2 + 2 -	4 +
3	ethyl ether	40	400	21.8	3.0	4	2 + 2 -	4 -
4	ethyl ether	40	400	21.8	24.0	4	2 + 2 -	4 -
5	ethyl ether	40	400		3.0	4	1 + 3 -	4 +
6	ethyl ether	40	400	4.1	3.0	4	1 + 3 -	4 +
7	ethyl ether	40	400	1.0	3.0	4	1 + 3 -	4 +
8	ethyl ether	40	400	24.0	3.0	4	1 + 3 -	2 + 1 (†) 1 -
9	ethyl ether	40	400	16.0	5.0	2	2 -	1 + 1 (†)
10	ethyl ether	40	400	8.25	10.6	3	3 -	1 (†) 2 -
11	ethyl ether	40	400	8.0	3.0	3	3 -	3 -
12	ethyl ether	40	400	4.0	3.0	2	2 -	2 -
13	ethyl ether	40	400	4.0	2.0	3	3 -	3 -
14	ethyl ether	150	250	4.0	2.0	3	3 -	3 -
15	methyl alcohol & pet. ether (1/1)	150	500	4.0	2.0	4	4 -	4 +
16	methyl alcohol & pet. ether (1/1)	150	500	4.0	4.0	2	2 -	2 -
17	methyl alcohol & pet. ether (1/1)	150	500	4.0	3.0	3	3 -	1 + 2 -

<sup>a</sup> (+) indicates presence of solvent flavor. Apparently, in the initial experiments some of the judges had difficulty in recognizing the solvent flavor since solvent flavor was sometimes reported in the fresh control. Results are included only to add weight to the results of the later experiments.

veloped by controlling the time of evacuation and the time of adsorption on coconut charcoal at liquid-air temperature. Although there are some inconsistencies

in the results reported in table 3, subsequent use has confirmed the conclusions reached in the time study that satisfactory removal of ethyl ether from at least 150 g. of butter oil was accomplished by 4-hr. evacuation with the pump at pressures of 0.1 mm. of mercury or less and 2-hr. adsorption on cocoanut charcoal at liquid-air temperatures, while the mixture of petroleum ether and methyl alcohol required an additional 2-hr. adsorption on the cocoanut charcoal.

The results in table 3 indicate that evacuation alone, even for 21.8 hr., is insufficient to reduce the solvent concentration in the butter oil below the threshold value. Apparently, pressures of solvent vapor of the order of 0.1 mm. of mercury are in equilibrium with a concentration of solvent in the butter oil which is above the threshold value. However, as also indicated by the data, some evacuation with the pump is necessary to reduce the total amount of solvent in the system below a certain value before the adsorption on the charcoal can reduce the concentration in the butter oil below the threshold value. The time of adsorption on charcoal required is apparently determined by the rates of diffusion of solvent in the various states and the dimensions of the apparatus.

#### THE REMOVAL OF STALE-FLAVOR COMPONENT WITH THE BUTTER OIL FROM THE STALE DRIED WHOLE MILK BY SOLVENT EXTRACTION

With the development of a special procedure for eliminating the interferences of solvent flavors with the judgments of the butter oil, a series of experiments was performed to determine whether the stale-flavor component was removed efficiently from the dried whole milk with the butter oil by solvent extraction.

*Experimental methods.* Three different procedures for pretreatment of the dried whole milk were employed, all of which had been found to yield satisfactory recoveries of butter oil on subsequent extraction with organic solvents. The conditions of extraction and solvent removal are supplied in table 4. After the solvent was removed from the extracted butter oils, they were blended with fresh skim milk to the composition of the original whole milk (4.3 per cent fat) and their stale-flavor threshold values were determined by the procedure described in our previous publication (4). Samples of the dried whole milk before pretreatment were reconstituted with distilled water to the same composition and their threshold values determined. These results also are recorded in table 4.

*Discussion.* Consideration of these results indicates that by all methods investigated the threshold value of the stale extracted butter oil is approximately the same as the percentage of fat from the stale reconstituted whole milk present at its threshold value.<sup>1</sup> Thus, it is indicated that the amount of the stale-flavor component per unit weight of fat is approximately the same for both the extracted butter oil and the dried whole milk from which it was extracted. Therefore, it can be concluded that, within the limits of experimental error, the efficiency of extraction of the stale-flavor component is proportional to the efficiency of extraction of the butter oil and that 90 per cent or more of the stale-flavor component is removed along with the butter-oil by the procedures used. In the

<sup>1</sup> Calculated by the following formula: The per cent fat is reconstituted whole milk times the threshold value of stale reconstituted whole milk.

first experiment, the presence of an off-flavor which was considered to be different from that of ethyl ether and which could not be removed by the solvent-removal technique indicated the possible formation of flavored reaction products

TABLE 4

*Effect of pretreatment and solvent extraction upon the efficiency of removal of stale-flavor component from dried whole milk*

	Expt. no.		
	1	2	3
Powder no.	104	104	104
Pretreatment	static hydration as in table 1, expt. 5	dynamic hydration as in table 1, expt. 6	alcohol treatment as in table 1, expt. 7, except time of treatment was 7 hr.
Moisture content after pre-treatment (%)	7.87	9.20	
Wt. of powder (g.)	176.8	198.5	205
Fat content of powder (%)	29.73	29.30	31.60
Solvent	anhydrous ethyl ether	pet. ether b.p. < 41 °C.	pet. ether b.p. < 41 °C.
Vol. of solvent (ml.)	750	850	850
Temp. of extraction (°C.)	40	43-49	47
Time of extraction (hr.)	4.25	23.75	9.17 <sup>a</sup> 4.17
No. of trips of syphon	19	18	18
Temp. of solvent removal (°C.)	40	40	47
Pressure during evacuation (mm. of Hg)	> 0.1 for 4 hr. < 0.1 for 4 hr.	< 0.1	> 0.1 for 1 hr. < 0.1 for 4 hr.
Time of evacuation (hr.)	8	20.2	5
Time of adsorption on charcoal (hr.)	2.25	4	4
Wt. of extracted butter oil (g.)	51.2	56.0	61.8
Recovery of butter oil (%)	97.5	96.2	95.4
Threshold value of stale reconstituted whole milk (%) <sup>b</sup>	(6) 40 ± 9	(6) 50 ± 9	(8) 50 ± 8
Fat from stale reconstituted whole milk at threshold value (%) <sup>c</sup>	1.7 ± 0.4	2.2 ± 0.4	2.2 ± 0.4
Threshold value of stale extracted butter oil (%) <sup>b</sup>	(4) 2.8 ± 0.9	(8) 1.8 ± 0.5	(6) 1.0 ± 0.5
Comments	Medicinal, Solvent Pyrolysis Product		

<sup>a</sup> The alcohol-treated powder was divided into 2 equal fractions and extracted in 2 different Soxhlet extractors. The difference in time of extraction is due to the design of the apparatus.

<sup>b</sup> The numbers in parenthesis represent the no. of judgments. Rejected judgments are not included.

<sup>c</sup> Calculated by the following formula: % Fat in reconstituted whole milk × threshold value of stale reconstituted whole milk.

from the ethyl ether and some component of the butter oil. Since this off-flavor was not present in the other experiments in which petroleum ether was used as the extracting solvent, it appears to be the more suitable solvent for this study.

## SUMMARY

In this study of the stale flavor which develops in dried whole milk on storage, it was necessary to develop a more efficient method for obtaining stale butter oil from the dried whole milk.

An investigation of various Soxhlet-type extraction procedures with organic solvents resulted in two suitable procedures which yielded better than 90 per cent recovery of stale butter oil.

Difficulties encountered in the removal of solvent from the extracted butter oil necessitated the development of a special technique to reduce the solvent concentration in the butter oil to the point where it did not interfere with the organoleptic judgment of the product.

The stale-flavor component was extracted with the butter oil by these procedures in approximately the same ratio to the fat as existed in the original dried whole milk and therefore may be considered to be better than 90 per cent extracted from the dried whole milk.

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# A NEW INDICATOR METHOD FOR THE DETERMINATION OF DIGESTIBILITY AND CONSUMPTION OF FORAGES BY RUMINANTS

J. T. REID, P. G. WOOLFOLK, C. R. RICHARDS, R. W. KAUFMANN, J. K. LOOSLI,  
K. L. TURK, J. I. MILLER AND R. E. BLASER

*Departments of Animal Husbandry and Agronomy, Cornell University*

The search for an indirect method for measuring the digestibility and consumption of feedstuffs by animals has been in progress for many years. A satisfactory procedure conceivably could eliminate the necessity of long, tedious and expensive digestion trials. In addition to determining the digestibility of dry and green feeds, an adequate method also would allow the indirect measurement of the quantity of pasture herbage consumed by grazing animals. Many other obvious applications of such a method are possible.

Most of the proposed methods require that certain reference substances used as "indicators" occur naturally in or be added to the feedstuff being examined. For the purpose of estimating consumption, it is essential that the reference substance be a normal constituent of the feed. Based upon the difficulties encountered in the use of various indicator methods it would seem that the ideal method should possess the following features: (a) It should employ a reference material which occurs naturally and in a measurable quantity in the feedstuff; which is indigestible and, therefore, completely recoverable in the feces; and, for which the chemical analysis is simple, accurate and rapid. (b) The recovery of the reference substance from the feces must not be influenced by treatment of the feed (curing methods, heat, etc.), by stage of maturity or by irregular passage of the "indicator" through the gut. (c) The equilibrium of the reference substance in the feces with that in the feed must be established soon after feeding is begun in order that short time trials may be used.

The authors are not aware of any reports giving previous attempts to employ natural plant pigments or chromogens<sup>1</sup> as reference substances. A summary of the voluminous literature dealing with the use of silica, iron oxide, chromic oxide and lignin as indicators will not be attempted in this report.

It was the object of this study to determine whether forages contain natural chromogenic substances which are indigestible, completely recoverable in the feces and, therefore, adaptable to use as a reference material for the indirect measurement of digestibility and consumption of forages by ruminants.

## EXPERIMENTAL PROCEDURE

In the first part of the investigation conventional digestion trials were con-

<sup>1</sup> For lack of better terms, "chromogen(s)" and "chromogenic substances" are employed throughout this report to refer to substances in solution absorbing light. Whether or not the substance(s) absorbing light at 406 mμ is a natural plant pigment or is chromogenic is not known.

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ducted with a mixed forage of which aliquots had been cured by ensiling and by drying in the field, barn and oven. All hays were fed to four wethers weighing 70 to 90 lb. in one replication of a  $4 \times 4$  Latin square, whereas silage and barn-dried and field-cured hays were fed to three bull calves weighing 350 to 375 lb. in one replication of a  $3 \times 3$  Latin square. No feed other than these forages was fed. Ten-day preliminary and fecal collection periods were employed. The forages fed and the resultant feces produced in these 25 trials provided the materials used in the first part of the study.

The ratio of the dry matter consumed in forage to that excreted in feces was established for each animal and forage in the conventional trial as a prerequisite to the pursuit of the problem. In order to ascertain whether any naturally occurring chromogenic substances present in the forages were completely recoverable in the feces, absorption measurements were made of acetone extracts of the forages and of their fecal products in the visible spectral range. Samples of the forages as fed and the resultant fresh feces were weighed in quantities proportional to the dry matter consumption-excretion ratio for a given animal. It is apparent that this procedure would allow the demonstration of indigestible substances should they exist. The samples were extracted in the same volume of 85 per cent acetone and the absorption spectrum was determined for each extract, using a Beckman DU model spectrophotometer. According to well established laws and assuming a minimum of interference, solutions of similar source absorbing the same quantity of light at a given wavelength theoretically contain equal quantities of the same chromogenic substance. Therefore, it follows that at wavelengths where equal quantities of light were absorbed by the forage and corresponding fecal extracts (disregarding the possible presence of interfering substances), some chromogenic substance(s) was present in both extracts in the same quantity. Furthermore, the presence of such isosbestic points would indicate that the chromogen(s) of forages responsible for the absorption of light at the corresponding wavelengths is indigestible and may be recovered completely from the feces. Upon determining the absorption spectra of the extracts of the different forages and corresponding feces samples it was found that an isosbestic point consistently existed near  $406\text{ m}\mu$ , as shown in figure 1. Although isosbestic points occurred at other wavelengths, the amount of light absorbed was undesirably low and in some cases appeared to be characteristic of a certain kind of forage. Since a high degree of absorption occurred at  $406\text{ m}\mu$ , indicating that a relatively large quantity of the chromogen(s) responsible for this absorption was present in the extracts, attention was largely given to this wavelength.

In order to employ these findings in an indicator technique for the measurement of forage digestibility and consumption, it was necessary, first of all, to quantitate the absorption measurements of the extracts in terms of a known chromogen at  $406\text{ m}\mu$ . Since the chromogen(s) responsible for absorption at  $406\text{ m}\mu$  was unknown and since the absorption maximum of  $\text{Na}_2\text{CrO}_4$  in aqueous solution is reasonably close ( $370\text{--}375\text{ m}\mu$ ) to  $406\text{ m}\mu$ ,  $\text{Na}_2\text{CrO}_4$  was employed for this purpose. Calibration of the spectrophotometer at  $406\text{ m}\mu$  was effected by

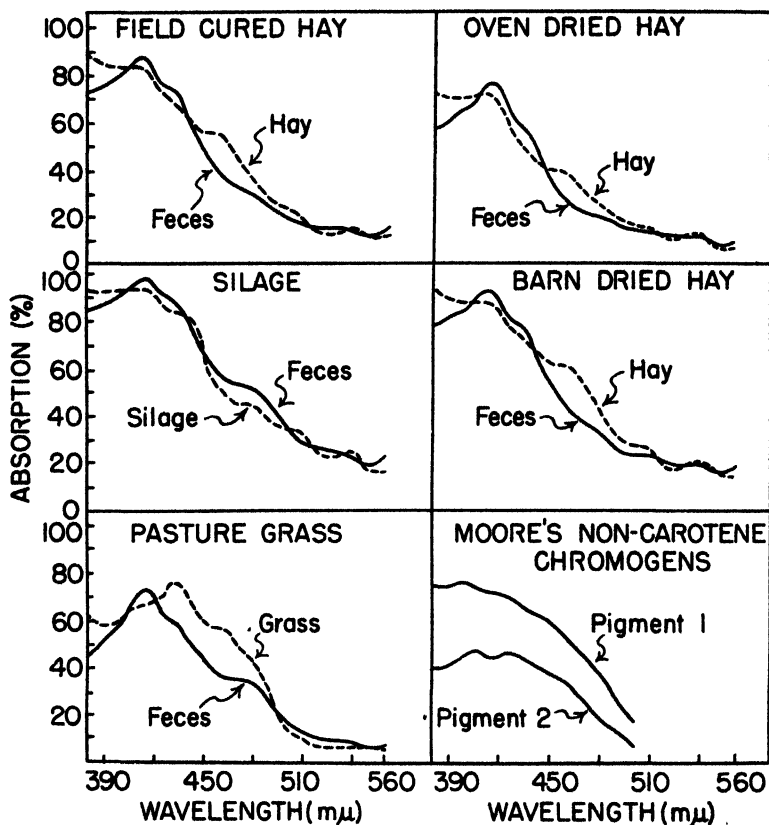


FIG. 1. Absorption spectra of acetone extracts of forages and of corresponding feces showing the formation of the isobestic point at 406 mμ. The spectra of the extracts of hays and silage and corresponding feces were obtained from the studies conducted with wethers, whereas those of pasture grass and its resultant feces were obtained using steers.

making absorption measurements on solutions of  $\text{Na}_2\text{CrO}_4$  ranging in concentration from 0 to 20 mg. per cent. The amount of light absorbed by a solution containing 1 mg. per cent  $\text{Na}_2\text{CrO}_4$  was termed equivalent to 1 unit of "chromogen" per 100 ml. of extract. For the particular instrument used in this study, the relationship of the concentration of chromogen in extracts to the quantity of light absorbed is expressed by the equation  $Y = 49.2379 - 23.8010 X$ , where  $Y$  = units of chromogen per 100 ml. of extract and  $X$  = log of the per cent of transmitted light.

Following these preliminary studies and as a second part of the investigation, the method finally adopted was tested further with hay containing a large proportion of Ladino clover. This was an attempt to determine whether the chromogen(s) under scrutiny were common to more than one plant species. Although the hays and silage used in the first part of the study were mixed, one plant possibly could have been the source of the pigment.

In addition to these trials, a study was made of the method's applicability to pasture grass. A known quantity of freshly clipped timothy-mixed grass forage was fed four times daily at 4-hr. intervals to three steers (two Holsteins and one Hereford weighing 375 to 500 lb.) confined in a barn and fitted with fecal collection bags. The pasture grass was studied at three timothy growth stages characterized as vegetative, boot to early head and full bloom, giving a total of nine trials. Simultaneously, total fecal collections were made from three other steers allowed to graze similar grass. Grass for analysis was sampled beginning 2 days prior to and during the first 2 days of fecal collection. The collection of feces was made over a period of 4 consecutive days. During the first day of the third trial the harness on one Holstein steer was damaged irreparably, and consequently, data were obtained on only two steers during this trial.

For all forages examined in these studies the analyses for chromogen(s) were conducted on feces as voided (undried) and on forage material in the same state as that fed to the animals.

All moisture measurements were made by the toluene distillation method. Care was taken to protect all samples and their extracts from light insofar as this was possible. All samples not in immediate process of analysis were kept in a refrigerator at 1 to 5° C.

*Details of Adopted Method.* Although the size of forage and feces samples convenient for extraction and the degree of dilution of the original extract will vary with the chromogen(s) content of the forage being studied, table 1 sum-

TABLE 1  
*Convenient sample weights, extract volumes and dilution rates*

Material	Approximate sample wt.	Vol. of original extract	Dilution of original extract
	(g.)	(ml.)	(diln. factor)
Hay, mixed grass	10-12	2000	2-3
Hay, Ladino	5-6	2000	2-3
Silage, mixed grass	20-25	2000	2-4
Pasture grass (largely timothy)			
vegetative stage	20	2000	2-3
boot to early head	20	2000	2-3
full bloom stage	20	2000	2
Feces of sheep fed:			
Hay, mixed grass	5-10	1000	3-5
Hay, Ladino	3-5	1000	5-8
Silage, mixed grass	4-6	1000	4-5
Feces of bulls and steers fed:			
Hay, mixed grass	6-10	1000	2
Silage, mixed grass	5-7	1000	2-3
Pasture grass			
vegetative stage	5-7	1000	5
boot to early head	5-7	1000	4
full bloom stage	5-7	1000	2-3

marizes the weights and volumes found to be practicable in this study.

Samples were weighed on filter paper of a diameter commensurate with the bulkiness of the sample and transferred with the paper to a 500-ml. boro-silicate



Waring blender cup equipped with a large rubber stopper covered with aluminum foil. A glass tube extending from the base of and through the stopper approximately 6 to 8 in. above the cup was found to prevent leakage from around the stopper caused by pressure due to increased temperature accompanying blending. Two hundred fifty to 400 ml. of 85 per cent (by volume) acetone (depending upon the final volume of extract desired) were added to the weighed sample and the blending was begun. The blender was allowed to run approximately 3 to 7 min. during which the cup was removed at intervals from the motor unit and placed in an ice-water bath. The number of times cooled during an extraction was determined by the degree of heating. The contents of the cup were transferred quantitatively to a Buchner funnel containing Whatman no. 42 paper, filtered by suction, and the macerate was washed with 85 per cent acetone. The residue then was returned to the blender cup and extracted in the same manner two or more times, depending on the degree of pigmentation of the successive extracts, toughness of the material being extracted and the fineness of maceration of the residue. Results were found to be readily reproducible when the final washings were clear and when stems were well macerated. Green timothy grass in the full bloom stage, clipped into short pieces with shears, was considerably more difficult to extract completely than the same grass at an earlier growth stage. More extensive treatment was required for this kind of grass than for any of the other materials extracted. Feces from pasture grass-fed steers was the most easily extracted material studied.

The extracts were made to a known volume and a portion of this sufficiently large to prepare a final dilution was filtered by gravity through Whatman no. 40 or 42 filter paper.

An absorption measurement then was made on the properly diluted extract at 406  $m\mu$ , using a Beckman spectrophotometer. The units of chromogen(s) per gram of dry matter were calculated according to the equation shown above. Substitution of the chromogen(s) values in the following equation allows the derivation of the apparent digestion coefficient for any nutrient or the dry matter without a knowledge of the total quantity of feces produced or of the forage consumed.  $\text{Apparent digestibility} = 100 - \left( 100 \frac{a \cdot x \text{ in feces}}{b \cdot x \text{ in forages}} \right)$ , where  $a$  = units of chromogen per g. forage,  $b$  = units of chromogen per g. feces and  $x$  = per cent of specific nutrient.

When the total yield of feces is known, the daily dry matter intake may be determined according to the following equation:  $\text{Dry matter consumption (g./day)} = \frac{(\text{units of chromogen(s) per g. dry feces}) \times (\text{g. of dry matter in feces per day})}{\text{units of chromogen(s) per g. dry matter in forage}}$

## RESULTS

The average rates of recovery from the feces of chromogen(s) absorbing light at 406  $m\mu$  for the forages studied are summarized in table 2. Table 3 sum-

TABLE 2

*Recovery at 406 m $\mu$  of chromogen(s) from feces of animals receiving various forages*

Forage	Experimental animals	Recovered in feces (%)	
		Av.	Range
Field-cured hay	Wethers (4) <sup>a</sup>	102.0 <sup>b</sup>	99.9-106.2 <sup>b</sup>
	Bull calves (3)	100.8	100.2-101.7
	All animals (7)	101.5 <sup>b</sup>	99.9-106.2 <sup>b</sup>
Barn-cured hay	Wethers (4)	99.0	96.2-100.3
	Bull calves (3)	101.1	98.4-104.2
	All animals (7)	99.9	96.2-104.2
Oven-dried hay	Wethers (4)	99.9	97.4-102.3
Silage, hay crop	Wethers (4)	98.6	96.4-100.7
	Bull calves (3)	100.8	99.0-102.6
	All animals (7)	99.5	96.4-102.6
Ladino clover hay	Wethers (2)	100.9	100.6-101.3
Pasture grass:			
vegetative stage	Steers (3)	100.9	97.2-102.4
boot to early head stage	Steers (3)	102.7	98.8-105.8
full bloom stage	Steers (3)	100.3	94.4-103.3

<sup>a</sup> Figures in parentheses represent the no. of animals used.<sup>b</sup> When the datum on one wether for which only one fecal analysis was made is disregarded, the av. and range of recovery become 100.7 (99.9-101.2) and 100.7 (99.9-101.7) % for wethers and all animals, respectively, on field-cured hay.

TABLE 3

*Av. dry matter digestion coefficients of forages estimated by the chromogen method as compared to those derived from conventional digestion trials*

Forages	Experimental animals	Dry matter digestibility (%)	
		Conventional trial	Chromogen method
Field-cured hay	Wethers (4) <sup>a</sup>	53.2 <sup>b</sup>	54.8 <sup>b</sup>
	Bull calves (3)	53.8	54.1
	All animals (7)	53.4 <sup>b</sup>	54.5 <sup>b</sup>
Barn-cured hay	Wethers (4)	53.3	52.7
	Bull calves (3)	55.2	56.0
	All animals (7)	54.1	54.1
Oven-dried hay	Wethers (4)	55.4	55.4
Silage, hay crop	Wethers (4)	48.2	47.4
	Bull calves (3)	52.6	53.0
	All animals (7)	50.1	49.8
Ladino clover hay	Wethers (2)	68.0	68.3
Pasture grass:			
vegetative stage	Steers (3)	72.9	73.3
boot to early head stage	Steers (3)	66.3	67.2
full bloom stage	Steers (3)	58.0	58.2

<sup>a</sup> Figures in parentheses represent the no. of animals used.<sup>b</sup> When the datum on one wether for which only one fecal analysis was made is disregarded, the average dry matter digestibility as determined by the conventional and chromogen(s) methods, respectively, becomes 53.3 and 53.6% for wethers and 53.5 and 53.9% for all animals on field-cured hay.

marizes the data for the digestion coefficients derived from conventional trials and from the chromogen(s) method, while figures for the actual intakes and those calculated from the chromogen(s) data are shown in table 4. These data

TABLE 4

*Av. daily dry matter intakes of forages estimated by the chromogen method as compared with the actual daily dry matter consumption*

Forage	Experimental animals	Dry matter intake (g./day)	
		Actual	Chromogen method
Field-cured hay	Wethers (4) <sup>a</sup>	454 <sup>b</sup>	461 <sup>b</sup>
	Bull calves (3)	3142	3166
	All animals (7)	1606 <sup>b</sup>	1620 <sup>b</sup>
Barn-cured hay	Wethers (4)	474	467
	Bull calves (3)	3402	3467
	All animals (7)	1729	1753
Oven-dried hay	Wethers (4)	443	445
Silage, hay crop	Wethers (4)	356	352
	Bull calves (3)	2627	2654
	All animals (7)	1330	1338
Ladino clover hay	Wethers (2)	712	719
Pasture grass:			
vegetative stage	Steers (3)	4077	4114
boot to early head stage	Steers (3)	4949	5095
full bloom stage	Steers (3)	4096	4112

<sup>a</sup> Figures in parentheses represent the no. of animals used.

<sup>b</sup> When the datum on one wether for which only one fecal analysis was made is disregarded, the av. quantity of dry matter consumed daily as determined by actual measurement and the chromogen(s) method, respectively, becomes 507 and 510 g. for wethers and 1825 and 1838 g. for all animals.

are not, in all cases, derived from duplicate measurements on feces and most of those showing the greatest deviation from perfect recovery were the result of a single determination. This was necessitated by the time required in working out certain details of the method, especially during the early stages. Therefore, it would seem that for forages similar to those examined in this investigation, absorption measurements on acetone extracts of forage and the resultant feces of animals consuming the respective forage will allow the estimation of digestion coefficients and dry matter consumption in close agreement with the actual (that derived from a conventional digestion trial), assuming that the usual care is taken in the conduct of the trial.

Equally good results were obtained with the proposed method when applied to a study of clipped pasture grass during three growth stages as when employed for the study of hays and hay-crop silage. The rates of recovery of the chromogen(s) from the feces of wethers, bull calves and steers were similar.

The mixed forage cured by four different methods ranked in descending order of chromogen concentration as follows: silage, oven-dried hay, barn-cured hay and field-cured hay. Although no extensive investigation has been made of the stability of the chromogen(s) studied, it was found to be light labile.

However, chlorophyll and carotene were lost more readily upon exposure to sunlight than the chromogen(s) responsible for the absorption at 406 m $\mu$ . No great loss of chromogen(s) was observed from extracts allowed to sit at room temperature in the dark from several days. Chromogen analyses made at intervals of a month on the same feces stored at 1 to 5° C. were in very close agreement. It is indicated that chromogen analyses do not have to be made on strictly fresh material. However, more extensive studies dealing with stability are in progress and may alter this general picture.

In a few cases where animals refused a small quantity of the forage offered, the chromogen content of theorts was much less than that of the forage offered. Even in the case of chopped hays, a marked degree of selective feeding was observed. This was indicated not only by the low chromogen level, but also by the low protein and high fiber content of the refused feed.

The digestibility and consumption of pasture grass dry matter by grazing steers, as estimated by the proposed method, are shown in table 5. These intake

TABLE 5

*Comparison of dry matter digestibility and consumption of pasture grazed at three growth stages as determined by the dry matter consumption-excretion ratio method and the chromogen(s) method*

Steer no.	Dry matter digestibility (%)		Estimated dry matter intake (g./day)	
	D.M.C.-E.R. method <sup>a</sup>	Chromogen method	D.M.C.-E.R. method <sup>a</sup>	Chromogen method
<i>Vegetative stage</i>				
0		79.1	3292	4267
36		76.8	5443	6350
39		75.8	4672	5227
Av.	72.9 <sup>b</sup>	77.2	4469	5281
<i>Boot to early head stage</i>				
0		74.8	3579	4787
36		65.0	5202	5003
39		68.2	3879	4103
Av.	66.3 <sup>b</sup>	69.3	4220	4631
<i>Full bloom stages</i>				
0		67.4	3135	4017
39		64.4	4041	4744
Av.	58.1 <sup>b</sup>	65.9	3588	4381

<sup>a</sup> Abbreviation for "dry matter consumption-excretion ratio" method.

<sup>b</sup> Dry matter digestion coefficients for clipped grass fed to "inside" steers as shown in table 3 are assumed to be the same for grass consumed by grazing steers when the dry matter consumption-excretion ratio method is employed. Therefore, these figures are recorded in this table in order that they may be readily compared to those obtained by the chromogen(s) method.

<sup>c</sup> Three grazing steers were begun in the study of grass at full bloom stage, but one steer was eliminated because of damage incurred to fecal collection bag harness.

estimates are somewhat higher than those determined by the simultaneous dry matter consumption-excretion ratio method. The digestibility of dry matter of the grazed grass was appreciably higher than that of the barn-fed clipped grass (table 3), indicating that grazing animals tend to select the more nutritive portions of the grass.

## DISCUSSION

The results indicate that it is feasible to use the chromogen(s) of forages absorbing light at 406  $m\mu$  as an "indicator" for digestibility and consumption measurements. The method as proposed is simple, rapid and accurate. It appears to be readily applicable to many kinds of ruminant nutrition experiments involving feeding trials, lactation trials and, especially, studies dealing with the evaluation of pastures in which a measure of consumption rate as well as digestibility is highly essential.

One weakness common to all methods employing naturally occurring "indicators" as applied to pasture studies is the inability to analyze grass truly representative of that selected by the grazing animal. If the substance used as the reference material was of the same concentration throughout the plant, the seriousness of the problem would be greatly minimized. However, the problem exists when either the lignin or chromogen ratio technique is applied. The lignin content of the leafy portions of the plant is much lower than that of the stems, whereas for the chromogen(s) measured at 406  $m\mu$  the reverse relationship was found. The data obtained in this study show that the intake of grazing steers estimated by the chromogen method is higher than that calculated on the basis of the dry matter consumption-excretion ratio which was determined for steers fed known quantities of similar grass. The quantity of dry matter consumed (estimated by chromogen method) by grazing steers was higher than that of similar steers fed a maximum level of clipped grass in the barn. However, it is doubtful that a difference as great as that observed actually existed. Since the level of chromogen(s) was higher in leaves than in stems and since the steers tended to select the leaves, probably because of greater palatability, the grass actually consumed by the steers probably contained a higher concentration of the chromogenic substance(s) than the analysis of clipped grass indicated. Therefore, a higher-than-actual intake was most probably the resultant estimate. This hypothesis would appear to be substantiated further by the fact that the chromogen(s) content of the grass refused by the barn-fed steers was much lower than that of the grass offered.

Data recently published by Forbes and Garrigus (1) show that the intake of steers estimated by the lignin ratio method generally is lower than that estimated by the dry matter consumption-excretion ratio technique. An explanation for this seems to be that the portion of the plant actually consumed by the animal is lower in lignin than the representative grass upon which the lignin analyses were made.

Regardless of the accuracy which may be demonstrated for the proposed method (chromogen), the lignin ratio or any other indirect method for evaluating forages when the material analyzed is the same as that fed, precise consumption data under grazing conditions is precluded at the present time by the inability to obtain samples of grass which are representative of that consumed by grazing animals.

An attempt was made in this study to confine grazing steers to an area which within 4 days would be grazed down to a level approximating the height of the stubble from which the grass was clipped for the barn-fed steers. Since the forage received by the barn-fed steers was sampled on an aliquot basis at each clipping (four times daily), plucked samples were taken at corresponding times from the area being grazed. These 16 samples, each weighing 165 g., were used to form a 4-day composite sample upon which moisture and chromogen analyses were conducted. The observed grazing habits of the steers pointed to a fallacy in this method of sampling. During the first 2 days, the steers grazed the leaves and, thereafter, the remaining stems. Judging from the degree of fill, the intake of these steers was greatest during the first day of grazing and was progressively less each successive day. However, the procedure used for obtaining the plucked samples did not allow for the supposedly reduced intake on successive days, *i.e.*, the samples taken were not proportional to the consumption of the steers. Since the chromogen level of plucked samples was considerably lower than that of clipped samples, a greater stem to leaf ratio existed in the plucked sample than in either the clipped sample or the grass actually consumed by the steers. Accordingly, the quantity of chromogenic material in the grass consumed by the barn-fed steers was used in calculating the data for the grazing steers.

The dry matter consumption-excretion ratio method originally suggested by Garrigus and Rusk (2) for use in pasture evaluation generally is considered to be the best method available for critical studies. However, the major recognized weakness of this procedure as applied to grazing studies is the assumption that the digestibility of the forage selected by grazing animals is the same as that of clipped grass fed to "inside" steers. The data derived by the chromogen method (table 5) for the pasture grass used in this study indicate that grazing animals select grass of a higher digestibility than that of grass from the same source but clipped and fed. Although the data are too few to allow definite conclusions, an examination of the body weight gains of the grazing steers indicates that the dry matter intake calculated by the chromogen method approaches more closely the probable intake based upon daily digestible nutrient requirements than do the estimates derived from the dry matter consumption-excretion ratio method. Therefore, these data suggest that the consumption estimates effected by the proposed method are more nearly comparable with production response than are the estimates made by the method suggested by Garrigus and Rusk (2). However, the chromogen(s) content of the grass consumed by grazing steers probably was higher than that resulting from the analysis of clipped grass, so the dry matter intake and digestibility estimates probably are somewhat higher than the actual figures.

Although the identity of the chromogen(s) employed in the proposed method is not essential to the execution of the method, it may be that a knowledge of its nature would lead to the improvement of the procedure. In addition, information in this connection would be of interest from a purely scientific standpoint. One possibility as to its identity is suggested by the spectral studies made of various non-carotene chromogens (fig. 1) extracted from dehydrated alfalfa meal

by Moore (3). The absorption spectra for the crude acetone extracts employed in the method proposed here resemble the spectra found by Moore for pigments 1 and 2 (in Skellysolve B) (fig. 1) which he had classified among a group of non-carotene chromogens. Moore (3) detected a very low vitamin A activity for these pigments used as a group in biological assays with rats. Whether or not pigments 1 and 2 were responsible for any part of the vitamin A activity found is not known.

Since the recovery of the pigment in the feces was so consistent for all forages studied, and since it would seem improbable that several chromogens consistently would exhibit an absorption relationship resulting in the formation of isosbestic points near  $406\text{ m}\mu$ , one chromogenic substance apparently is responsible for the absorption observed. However, since acetone is a polar solvent, combination of the solvent with some substance to form a chromogen capable of absorption at  $406\text{ m}\mu$  is possible. Further study employing non-polar solvents to examine this possibility is planned.

*Sources of Error.* The errors inherent in the conventional digestion trial appear to be common to this method also. In addition, analytical errors are possible; the major one of these experienced during the investigation was incomplete extraction of the pigments from certain kinds of samples. Mature, tough timothy-mixed grass was considerably more difficult to extract completely than the same kind of grass at an early growth stage, or than finely ground hay samples. Feces, probably because of maceration by the animal, were extracted more easily than forage materials. However, sheep feces, especially those containing in excess of 60 per cent dry matter, were more difficult to extract than those of bull calves or steers. Sampling errors were believed to be of the greatest magnitude with silage and with the green fresh grass clipped to approximately 0.5 in. lengths before weighing. Samples weighing approximately 20 g. were employed to aid in counteracting this error. In an attempt to arrive at a representative chromogen figure on green grass, six or more analyses were made. This problem appeared to be minimized with regard to finely ground hays and feces as excellent agreement was found in most cases in the analysis of duplicate samples. The blending of a sample in acetone to a temperature where acetone is being lost (reducing the ratio of acetone to water) poses a problem which was not explored. This could be an error in that it may influence the absorption of light by a given extract. However, this was controlled, as far as possible, by cooling the blended samples at intervals in an ice-water bath. The  $\text{Na}_2\text{CrO}_4$  solutions used as standards may respond to the Beer-Lambert law at  $406\text{ m}\mu$  in a manner different from that of the chromogen(s) responsible for the absorption measured at this wavelength and thus cause errors. However, the rates of recovery observed in this study would justify the use of  $\text{Na}_2\text{CrO}_4$  in this way. It is the desire of the authors to attempt the isolation of the chromogen(s) in at least a crude form and examine the feasibility of its use in the calibration of the method.

## SUMMARY

Mixed forage of the same source cured by barn, oven and field drying and by ensiling, hay consisting largely of Ladino clover, and pasture grass (largely timothy) at three different growth stages were fed to wethers and/or bull calves and/or steers in 36 conventional digestion trials. The dry matter digestibility of these forages ranged from 48.2 to 72.9 per cent.

Spectral examinations of acetone extracts of the forages studied and their corresponding fecal products revealed that some chromogen(s) absorbing light at  $406\text{ m}\mu$  was completely recoverable in the feces. The average rate of recovery of the chromogenic substance(s) in the feces of animals fed the forages used in these studies was 100.5 per cent for the 36 trials.

As a result of these studies, a simple, accurate method employing the chromogen(s) absorbing light at  $406\text{ m}\mu$  as a reference substance was devised for the estimation of digestibility and consumption of forages by ruminants. This method apparently has a wide range of applicability in nutrition studies with ruminant animals, especially in the study of pastures where a direct measure of consumption is impossible.

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## THE ACCURACY OF LINEAR BODY MEASUREMENTS OF DAIRY CATTLE<sup>1</sup>

R. W. TOUCHBERRY<sup>2</sup> AND J. L. LUSH  
*Iowa Agricultural Experiment Station, Ames*

Measurements such as weight, milk production, egg production, speed and linear body measurements are often used in animal husbandry for determining progress of breeding methods or the effects of different rations. Such measurements can have a high degree of objectivity and are adaptable to statistical analyses. The validity of the conclusions drawn from the statistical analyses depends partly on the accuracy of the original measurements. The present study is concerned with the amount of random error in linear body measurements of dairy cattle.

Phillips and Dawson (7) concluded that measurements taken directly from hogs were more accurate and required less time than obtaining measurements from photographs or by a livestock scaling stick. Phillips and Stoeck (8) found that measurements taken directly from sheared sheep generally were more accurate than those obtained from photographs. By 11 repetitions of 25 measurements on each of nine Jersey cows and ten Jersey heifers, Lush and Copeland (5) found that there was little or no correlation between the average size of the measurement and the random error in taking that measurement. Single observations of measurements were, in most cases, accurate enough to obtain significant differences between animals, but averages based on two or more repetitions of the measurements were more accurate. On comparable measurements the absolute sizes of the errors they found were approximately of the same order as those in the present study.

Lush, *et al.* (4), in studying weights of cattle, concluded that the average of 3-day weights was not absolutely accurate, but the random error was only 57 per cent of that of 1-day weights. Bean (2) and Baker *et al.* (1) found that single-day weights were more reliable than 3-day weights, but this contradictory finding can be attributed to the grouping of their data. They grouped their data into subgroups on the basis of the weights on the first day. The range within these groups on the first day was thus fixed within bounds, but these same groups could, on the second and third days, contain weights above or below the limits set for the group. This automatic effect of the method of grouping was mainly responsible for the contradictory conclusions that single-day weights are more accurate than the average of 3-day weights. Concerning weights of steers, Patterson (6) found that by two extra weights the mean square between animals could be reduced by only 0.65, 2.44 and 0.95 per cent in three groups of data. From this, he concluded that using 11 animals with single-day weights was more

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<sup>2</sup> Now Assistant Professor of Dairy Science, University of Illinois.

efficient than using 10 animals with 3-day weights, as judged by the standard error for animals.

The general conclusion from such studies is that extra weighings and measurements result in more accurate data, but there is a question from case to case as to whether the gain in precision is enough to warrant the extra trouble, time and expense of taking the extra data.

#### SOURCE AND DESCRIPTION OF DATA

The data for this study were from the Iowa State College Holstein herd from 1931 to 1946. Five characteristics, *i.e.*, wither height, chest depth, body length, heart girth and paunch girth, were measured three times at each of the ages, 6 mo. and 1, 2, 3, 4, 5 and 7 yr. The measurements were recorded to the nearest millimeter. The paunch and heart girth measurements were taken with a steel tape in a plane perpendicular to the body axis at the largest and smallest circumferences of the barrel, respectively. Chest depth, wither height, and body length were measured with calipers. Wither height was the vertical distance from the highest point over the withers to the ground. Chest depth was the vertical distance from the back to the floor of the chest at the shallowest part of the chest. Body length was the horizontal distance from the point of the shoulder to the end of the pin bones.

One measurement of each characteristic was made and recorded as the "first order" measurement. The animal then was moved to a new position and each characteristic was measured again; this was recorded as the "second order" measurement. The "third order" measurements were taken after the animal had been moved again to a new position. This procedure was followed at each of the seven ages at which measurements were taken. Care was taken each time to have the animal in a natural position standing rather squarely on all four legs. At the beginning of the project one man took most of the measurements, but in later years three men took the measurements so that one man would make only one measurement of a characteristic on each animal at each age. Beginning in 1943, the name of the man who took each measurement was recorded so that the effect of possible differences in the way various men used the measuring instruments could be measured.

#### ANALYSIS OF DATA

*Estimating the variance components.* Suppose  $P$  cows were each measured three times for each characteristic studied. Now, if the order in which each of the three measurements was taken is known, the data for each characteristic can be classified according to two criteria, namely, by cow and by order. The mathematical model for the data would be of the form

$$Y_{ijk} = \mu + A_i + B_j + X_{ijk} \quad (1)$$

where  $Y_{ijk}$  is the observed value of a characteristic on the  $i$ -th cow for the  $j$ -th order;  $\mu$  is the mean of all observed values,  $A_i$  is the amount by which the  $i$ -th cow is above or below the general mean.  $B_j$  is the amount the  $j$ -th order differs from the general mean, and  $X_{ijk}$  is the error associated with the  $Y_{ijk}$ . The  $X_{ijk}$

are caused by such things as mistakes in reading the instruments, differences in the pressure with which the instruments were applied, genuine changes in the animal such as taking in and letting out breath, interactions between orders and cows and many other things which are not controlled easily.

If now 3P equations of type (1) are written in place of the observed values, the measurements are expressed in terms of the components on which interest is centered. The sizes of the cow, order and error components of variance were estimated by an analysis of variance an example of which is shown as table 1. The sums of squares for total, for cows and for orders were calculated in the usual way as given in Snedecor (11). The error sum of squares was estimated by subtracting the sums of squares for cows and for orders from the total.

The differences between cows were significant far beyond the 0.01 level of probability for each characteristic and at each of the seven ages. This was expected because of the wide variation among the animals. The differences

TABLE 1  
*Analysis of variance for paunch girth of 3-yr.-old cows*

Source	Sum of squares	Degrees of freedom	Mean square	Expected mean squares	Estimated variance component
Total	97,181.38	731			
Cows	96,416.83	243	396.78**	$\sigma^2E + 3\sigma^2C$	$\sigma^2C = 131.74$
Orders	3.76	2	1.88	$\sigma^2E + 244\sigma^2D$	$\sigma^2D = 0.002$
Error	760.79	486	1.57	$\sigma^2E$	$\sigma^2E = 1.57$

\*\* Significant at the 0.01 level of probability.

between orders were significant at the 0.01 level of probability in 19 of the possible 35 cases and significant at the 0.05 level in two more cases, although the components for orders were too small to be important practically. Sixteen of the 19 highly significant cases were at the four youngest ages. Perhaps nervousness of the younger animals contributed to the order differences among them. Order differences also are partially confounded with differences between the men taking the measurements. Differences between the mean measurements by different men on the same animal were too small to have been important practically although, apparently, they were statistically significant in some cases. The details of this analysis are not shown since the man differences were confounded completely with the order differences until the last few years and were confounded partially with them then. Table 2 shows the cow, order and error components of variance for each characteristic at each age. The order and error components are very small when compared with the cow components.

*Relative value of one, two, and three measurements of a characteristic.* So far as the sizes of the variance components  $\sigma^2c$  and  $\sigma^2e$  in table 2 show correctly the variance from these causes, the relative value of measuring a characteristic one, two, three or  $k$  times can be estimated by the formula:

$$\text{Relative Value} = \frac{\sigma^2c}{\sigma^2c + \frac{\sigma^2e}{k}}$$

This formula is used with the assumption that the cows are a random sample, as they would be in most breeding experiments. As  $k$  becomes larger the relative value of the estimate approaches 1.00. When  $k = 1$ , the relative value of the estimate is at its lowest point and the relative value is the same as the intraclass correlation. Table 3 gives the relative values of one, two and three measurements of each characteristic at each age. One measurement of each characteristic seems accurate enough for most purposes. A possible exception is body length, for which estimates based on single measurements have relative values of 0.834 to 0.907, but estimates based on three measurements range from 0.936 to 0.967.

The relative values of the measurements are approximately the same for the same characteristic at the different ages. If the relative values for the various

TABLE 2  
*The components of variance for each characteristic at each age*

Age and no. of cows	Component <sup>a</sup>	Characteristic				
		Wither ht.	Chest depth	Body length	Heart girth	Paunch girth
6 mo. 367	C	14.98	4.85	24.86	33.26	78.19
	D	0.03	- trace	0.03	0.06	0.09
	E	0.57	0.21	2.59	0.93	1.43
1 yr. 348	C	13.73	5.80	29.06	49.80	85.33
	D	0.05	- trace	0.04	0.06	0.08
	E	0.76	0.45	3.00	1.18	1.66
2 yr. 329	C	11.39	5.27	21.40	54.52	136.03
	D	0.02	0.01	0.20	0.08	0.03
	E	0.71	0.40	3.92	1.81	1.37
3 yr. 244	C	13.26	6.26	22.09	54.58	131.74
	D	0.01	- trace	0.10	0.08	trace
	E	0.77	0.38	4.10	2.09	1.57
4 yr. 161	C	15.06	4.99	21.22	51.47	143.83
	D	0.02	0.01	- 0.02	0.04	0.01
	E	0.73	0.34	3.54	1.17	1.83
5 yr. 108	C	16.52	6.43	25.67	53.08	108.07
	D	0.03	trace	- 0.01	0.06	- 0.01
	E	0.67	0.30	3.49	2.04	1.77
7 yr. 38	C	10.91	3.66	25.66	64.09	165.36
	D	0.01	0.01	- 0.13	0.01	- trace
	E	0.57	0.41	5.11	1.71	2.16

<sup>a</sup> C = Cow component of variance.

D = Order component of variance.

E = Error component of variance.

ages of one characteristic are ranked in numerical order of their size as 1, 2, 3, . . . 7, a rectangular distribution is obtained. If there is no difference between the relative values at the different ages, the sums of the ranks at the seven ages should be approximately equal, and a chi square test of the mean ranks of the seven ages would detect any age difference in the relative values. Table 4 gives the ranks; the number of ranks is seven, corresponding with the number of ages, and the number of sets of ranks is five, corresponding to the five characteristics. In cases of ties, the relative values were assigned the average value of the ranks for which they were tied. A discussion of this method is given by Friedman (3).

TABLE 3

*Relative values of estimates based on 1, 2, and 3 measurements of each characteristic at each age*

Age	No. of repetitions	Characteristic				
		Chest depth	Wither ht.	Body length	Heart girth	Paunch girth
6 mo.	1	0.958	0.963	0.906	0.973	0.982
	2	0.978	0.981	0.951	0.986	0.991
	3	0.986	0.987	0.966	0.991	0.994
1 yr.	1	0.929	0.948	0.907	0.977	0.981
	2	0.963	0.973	0.951	0.988	0.990
	3	0.975	0.982	0.967	0.992	0.994
2 yr.	1	0.929	0.941	0.845	0.968	0.990
	2	0.963	0.970	0.916	0.984	0.995
	3	0.975	0.980	0.942	0.989	0.997
3 yr.	1	0.943	0.945	0.844	0.963	0.988
	2	0.971	0.972	0.915	0.981	0.994
	3	0.980	0.981	0.942	0.987	0.996
4 yr.	1	0.937	0.954	0.857	0.978	0.987
	2	0.963	0.976	0.923	0.989	0.994
	3	0.978	0.984	0.947	0.992	0.996
5 yr.	1	0.956	0.961	0.880	0.963	0.988
	2	0.977	0.980	0.936	0.981	0.994
	3	0.985	0.987	0.957	0.987	0.996
7 yr.	1	0.900	0.951	0.834	0.974	0.987
	2	0.947	0.975	0.900	0.987	0.994
	3	0.964	0.983	0.936	0.991	0.996

Using a modification of Friedman's formula, a chi square value of 5.35 with six degrees of freedom was found for testing the significance of differences in the ranks. Since this has a probability of 0.5, one can say that there is no indication of a difference in the relative accuracy of the measurements of the characteristics at the different ages. There also was no difference in the cow components of variance at the different ages. This method of ranking would not pick out an interaction between age and characteristic. Friedman's formula was modified only to the extent necessary to allow for having used fractional values where there were ties. The use of fractional values changes the sum of the squares of the first  $p$  integers.

TABLE 4

*Ranks of the relative values for each of the characteristics*

Characteristic	Age						
	6 mo.	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.	7 yr.
Wither ht.	7	2.5	2.5	5	4	6	1
Chest depth	7	3	1	2	5	6	4
Body length	6	7	3	2	4	5	1
Heart girth	4	6	3	1.5	7	1.5	5
Paunch girth	2	1	7	5.5	3.5	5.5	3.5
Sum of ranks	26	19.5	16.5	16.0	23.5	24	14.5
Mean rank	5.2	3.9	3.3	3.2	4.7	4.8	2.9
Deviation from mean rank	1.2	-0.1	-0.7	-0.8	+0.7	+0.8	-1.1

It was not necessary to make a chi square test of the ranks of the characteristics within each age to show that there was a highly significant difference between the relative accuracy value for the five characteristics. Paunch girth was the most accurate and was followed by heart girth, wither height, chest depth, and body length in that order. The ranks were in the same order at each of the ages.

*The error standard deviations and coefficients of variation.* Unless otherwise specified, the standard deviations referred to in this section are those due to errors of measuring. The coefficients of variation referred to are those found by expressing the error standard deviation as a percentage of the mean. Table 5

TABLE 5  
*The mean, error standard deviation and coefficient of variation for each characteristic at each age*

Characteristic	Age						
	6 mo.	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.	7 yr.
Wither ht. S.D.*	0.76	0.87	0.84	0.88	0.86	0.82	0.75
$\bar{X}$	98.9	114.9	129.3	133.6	135.3	136.1	138.4
C.V.	0.77	0.76	0.65	0.66	0.63	0.60	0.54
Chest depth S.D.	0.46	0.67	0.63	0.62	0.58	0.55	0.64
$\bar{X}$	46.1	57.0	68.5	71.3	73.3	74.5	71.9
C.V.	1.01	1.17	0.92	0.86	0.79	0.73	0.88
Body length S.D.	1.61	1.73	1.98	2.02	1.86	1.87	2.26
$\bar{X}$	105.9	128.7	151.0	158.9	163.3	165.4	167.6
C.V.	1.52	1.35	1.31	1.27	1.15	1.13	1.35
Heart girth S.D.	0.97	1.08	1.34	1.45	1.08	1.43	1.31
$\bar{X}$	119.5	148.2	181.1	186.3	191.3	194.2	202.2
C.V.	0.81	0.73	0.74	0.78	0.57	0.73	0.65
Paunch girth S.D.	1.20	1.29	1.17	1.25	1.35	1.33	1.47
$\bar{X}$	147.0	178.5	221.5	230.3	237.1	240.2	248.6
C.V.	0.82	0.72	0.53	0.54	0.57	0.55	0.59

\* S.D. and  $\bar{X}$  are expressed in cm.

gives the standard deviation, the mean and the coefficient of variation for each characteristic at each of the seven ages. The standard deviations at 6 mo. seem to be a little smaller than those at the other ages. On ranking the standard deviations within each row in table 5 and then comparing the columns, a chi square of 10.56 was found. With six degrees of freedom this chi square is significant at the 0.11 level of probability. Most of the small age difference seems only to reflect the fact that the standard deviations at 6 mo. of age are smaller than those at other ages.

The coefficients of variation are largest at 6 mo. and 1 yr. A chi square test of the mean ranks at the various ages gave a value of 18.15. With six degrees of freedom this is significant at the 0.01 level. This difference comes mainly from the large coefficients of variation at the first two ages. The coefficients of variation were smallest for paunch girth. Next in order came wither height, heart girth, chest depth and body length.

*How random errors and rounding affect correlations.* In animal breeding it is often necessary to correlate one characteristic with another. If  $\sigma^2 X_o$  and  $\sigma^2 Y_o$  are the observed variances of two characteristics  $X$  and  $Y$ ,  $\sigma^2 X_o$  and  $\sigma^2 Y_o$  the independent error variances involved in measuring the two characteristics and  $\sigma^2 X$  and  $\sigma^2 Y$  the true variances of the two characteristics, then  $\sigma^2 X_o = \sigma^2 X + \sigma^2 X_e$  and  $\sigma^2 Y_o = \sigma^2 Y + \sigma^2 Y_e$ . Shewhart (10) has shown that the true correlation between  $X$  and  $Y$  is  $\frac{\sigma X_o \sigma Y_o}{\sigma X \sigma Y}$  times as large as their observed correlation. In studying body measurements, if error variance,  $\sigma^2 E$ , and the variance due to differences between cows,  $\sigma^2 C$ , are known, the amount the random errors of measuring affect correlations can be shown. For example, if one were studying the correlation between chest depth,  $X$ , and wither height,  $Y$ , of 3-yr. old cows with the present data, the correlation between the observed average measurements would be  $\frac{\sigma X \sigma Y}{\sigma X_o \sigma Y_o} = \frac{(2.502) (3.641)}{(2.527) (3.676)} = 0.9807$  as large as the true correlation.

In working with any continuously distributed variable, some rounding must be done. Sheppard (9) long ago showed that the variance component for rounding is  $1/12$  where unity is the width of the grouping class; this is widely known as "Sheppard's correction". In this study the measurements were rounded to the nearest millimeter, and this error of rounding was included in the error of measuring. If the average measurements of the characteristics are rounded to the nearest centimeter, the variances of these averages would be increased by the amount  $1/12$ . The observed variances would now include the cow component, the error component and a component,  $1/12$ , due to rounding. The observed correlation between the rounded averages of the chest depth and wither height measurements of 3-yr.-old cows would be  $\frac{(2.502) (3.641)}{(2.543) (3.687)} = 0.9716$  as large as the true correlation. In these data random errors of measuring and errors of rounding to the nearest centimeter would have little effect on correlations between the body measurements.

#### DISCUSSION

The error components of variance did not change significantly with age and they would be affected very little by the heterogeneity of the group studied. In studying body measurements one would expect to find the following error standard deviations (in cm.): wither ht., 0.75 to 0.88; chest depth, 0.46 to 0.67; body length, 1.61 to 2.26; heart girth, 0.97 to 1.45; and paunch girth, 1.17 to 1.47. The cow components of variance in this study are for random samples at specific ages and for one breed and are smaller than cow components from random samples that would include all ages and different breeds. The cow component is thus dependent on the heterogeneity of the group studied, but in general, one would expect cow components of intra-breed variance to be of the order of those found in this study. Consequently, one measurement of each characteristic except body length is accurate enough for practical purposes, as

the cow components are so much larger than the error components. Two or three measurements of body length give approximately the same accuracy as single measurements of the other four characteristics. Even though single measurements are accurate enough for practical purposes, they do not provide a check on gross errors such as misreading a measurement by 10 or more cm. Duplicate and triplicate measurements provide a check on such gross errors.

In working with a more homogeneous group as found in some feeding experiments, the cow components of variance would be much smaller than those in this study. Consequently, the errors of measuring would be a relatively greater source of trouble as they presumably wouldn't change in absolute size. Under such conditions one might be justified in taking two or three measurements of each characteristic to obtain more accuracy as well as to check for gross errors.

Regarding conclusions drawn from measurements subject to random errors of measuring, Tryon (12) says, "When a difference between groups is empirically found, that difference is more significant in proportion as the measuring device is less reliable." Woods (13) says, "The worse we think the material the more certain we may be of our conclusions, provided there is no bias in favor of the results." Tryon's statement implies that the difference necessary for significance increases as the random error of measuring increases and, as Woods intimates, if there is a significant difference even when a large random error is involved, conclusions are more certain. Woods' statement applies only to the case where the hypothesis that the samples involved are from the same population is rejected; there would be less certainty in accepting the hypothesis.

#### SUMMARY AND CONCLUSIONS

The cow, order and error components of variance for the five body measurements, *i.e.*, wither height, chest depth, body length, heart girth and paunch girth, were observed at each of the seven ages, 6 mo., 1, 2, 3, 4, 5 and 7 yr. There were 367 animals measured at 6 mo., 348 at 1 yr., 329 at 2 yr., 244 at 3 yr., 161 at 4 yr., 108 at 5 yr., and 38 at 7 yr. Measurements were in centimeters.

In all cases, the order and error components of variance were very small as compared to the cow components. The largest error components were found in measuring body length. There they ranged from 2.59 to 5.11, while the cow components for body length ranged from 21.22 to 29.06. The smallest errors were for chest depth and these ranged from 0.21 to 0.45, while the cow components ranged from 3.66 to 6.43. Although significant in some cases, the order components were too small to be of practical importance.

The relative accuracies of the measurements of the characteristics were high. Paunch girth was the most accurate, for single measurements had a mean relative accuracy of approximately 0.986, while the corresponding figure for heart girth was 0.971. Single measurements of wither height and chest depth had relative accuracies of 0.952 and 0.936, respectively. Body length was least accurate, for single measurements had a relative accuracy of 0.866, but the average of three measurements brought the relative accuracy up to 0.951. There was no signifi-



cant difference between the relative accuracies at the seven different ages. The relative accuracies of the five characteristics did differ among each other with high statistical significance.

The error standard deviations did not increase significantly with the age of the animal, but the coefficients of variation were larger at the two youngest ages than at the older ages. The five characteristics differed significantly from each other in error standard deviations and in their coefficients of variation.

The random errors of measuring and also the errors of rounding make the observed correlations between body measurements smaller than the true ones, although this effect was negligible in the present study. Using the average of three measurements and rounding each average to the nearest centimeter reduced the correlation between chest depth and wither height of 3-yr.-old cows to 0.972 of what it would have been if there had been no errors of rounding or measuring. If the averages had been taken to the nearest millimeter the correlation would have been 0.981 as large as the true correlation.

It is concluded that single measurements are accurate enough for most practical purposes provided one can be sure that no gross errors such as reading a measurement 10 cm. too large or too small go undetected.

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# PARTURIENT PARESIS. V. BLOOD SERUM LEVELS OF CITRIC ACID AND CALCIUM IN NORMAL PARTURIENT COWS AND COWS WITH PARTURIENT PARESIS<sup>1</sup>

T. H. BLOSSER<sup>2</sup> AND VEARL R. SMITH

*Department of Dairy Husbandry, University of Wisconsin*

In recent years evidence has been accumulating that citric acid plays an important role in calcium metabolism in the body. Dickens (3) found that the hard substance of bone contained a large store of citric acid. Nicolaysen and Nordb (8) have reported that lack of vitamin D results in a decrease in bone citric acid content. Shohl (10) has demonstrated the favorable effect of oral administration of citrate buffer in the prevention and cure of rickets in rats on a rachitogenic diet. The effect is not due solely to the alteration of the pII of the intestinal tract.

The relation of citric acid to calcium excretion and urinary calculus formation has been studied by Shorr *et al.* (11, 12) and by Kissin and Locks (6). These investigators have demonstrated a decreased citric acid excretion with urinary calculi, and suggest an increased intra-renal citrate oxidation in this disease. Gomori and Gulyas (4) were able to increase urinary excretion of calcium in puppies by injecting 8 to 30 ml. per kg. of body weight of a 4 per cent solution of sodium citrate. The blood calcium remained essentially unchanged. Marek *et al.* (7) administered sodium citrate intravenously to cows and produced an 8 per cent increase in calcium excretion, chiefly via the kidneys.

That certain hormones may bear some relation to citric acid metabolism has been pointed out by several investigators. Alwall (1) injected parathormone intramuscularly into dogs and produced an increase in blood serum calcium from a normal level of 11.1 mg. per cent to 15.6 mg. per cent 13 hr. after injection. The corresponding rise in blood serum citric acid over the same time interval was from 76.5  $\gamma$  per ml. to 94.7  $\gamma$  per ml. Both calcium and citric acid had returned to normal 36 hr. post injection. Gomori and Gulyas (4) histologically observed the bones of puppies into which sodium citrate was injected and commented on the similarity of the lesions to those produced by injection of parathyroid extracts.

Shorr *et al.* (11, 12) have established a relation between certain of the steroidal reproductive hormones and citric acid excretion. This work demonstrated for the first time a very interesting relationship between estrogen and androgen production, citric acid metabolism and urinary calculi.

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<sup>1</sup> These data were taken from a thesis presented by the senior author to the faculty of the Graduate School, University of Wisconsin, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> Present address, Division of Dairy Husbandry, the State College of Washington, Pullman.

Inasmuch as levels of the steroidal reproductive hormones fluctuate markedly around the time of parturition Grollman (5) and since relationships between calcium and citric acid and between estrogens and citric acid have been demonstrated, a study was undertaken to determine the relationship between blood citric acid levels with parturient paresis and normal parturitions.

#### EXPERIMENTAL PROCEDURE

This experiment was conducted in three herds and covered a period of time extending from February 1947 to December 1948.

The initial phase of the study was conducted in the Jersey herd of Biltmore Farms at Biltmore, North Carolina. This was done in conjunction with the study on the effect of prepartum milking on the incidence of parturient paresis or milk fever reported by Smith and Blosser (13). All cows in this initial phase were prepartum milked. A total of 18 Jersey cows was studied in this phase, of which two developed milk fever.

The second phase of the study was done in the dairy herd of the University of Wisconsin. Six Guernsey, four Holstein and nine Jersey cows were studied. Five of the nine Jersey cows developed parturient paresis and one was prepartum milked.

The third phase of the study was made in the dairy herd of the State College of Washington. Four Jersey cows were used in this phase and one of them developed milk fever.

Cows in the second and third phases were handled in accordance with accepted management and feeding practices at the time of calving. Calves were permitted to nurse and cows were not milked completely dry for the first 48 hr. postpartum.

Blood samples were drawn daily, generally 3 to 5 days prior to parturition and from 3 to 5 days subsequent thereto; a few cows samples were obtained as early as 10 days prepartum and as late as 10 days postpartum. Ordinarily, samples for a given cow were drawn at the same time each day. In the studies made at Biltmore Farms, blood serum was sent in refrigerated cartons to Madison, Wisconsin, three times weekly for analysis.

Blood serum citric acid was determined by the method of Perlman *et al.* (9) in the first two phases, and by the method of Taussky and Shorr<sup>3</sup> (14) in the third phase. The latter method was somewhat more sensitive and reproducible with the small amount of blood serum used, but over-all results of the two methods were essentially the same. Blood serum calcium was determined by the method of Clark and Collip (2).

Cows were considered as calving normally if they did not exhibit gross clinical symptoms of any disease at calving. A few of the so-called normally calving cows were on the verge of parturient paresis, as is evident from their blood picture.

#### RESULTS AND DISCUSSION

The data obtained in this experiment are summarized in table 1, which presents mean levels of blood serum calcium and citric acid at different times pre-

<sup>3</sup> The *n*-heptane used in citric acid analyses by the method of Taussky and Shorr was kindly furnished by the Phillips Petroleum Co., Bartlesville, Okla.

TABLE 1  
*Mean levels of calcium and citric acid in the blood serum of normally calving and parturient paresis cows previous and subsequent to parturition*

		10	5	4	3	2	1	D. of parturition		1	2	3	4	5	10
Normally calving cows	No. of Analyses	4	13	11	15	22	27	31	31	31	29	28	9	8	2
	Calcium (mg. %)	11.0	10.3	10.3	10.2	10.0	10.3	8.7	8.4	8.4	9.6	10.1	10.5	10.7	11.0
	Citric Acid (mg. %)	6.62	5.46	7.17	7.20	6.92	6.24	4.70	3.73	4.93	5.64	5.66	5.98	4.78	
Parturient paresis cows	No. of Analyses	3	2	4	3	5	7	9	9	9	9	7	3	5	3
	Calcium (mg. %)	11.1	10.7	10.6	10.5	11.4	10.6	6.9	6.8	6.8	6.7	9.2	9.9	10.4	10.9
	Citric Acid (mg. %)	5.05	5.86	5.04	7.57	9.48	8.63	3.86	2.20	2.29	2.29	2.66	7.08	4.82	5.80

and postpartum. In computing the means, only those days were used where figures for both calcium and citric acid levels of the blood serum were available.

In analyzing the data, the question arose whether the normally calving cows of all three breeds and both prepartum- and non-prepartum-milked cows could be used to compare to the milk fever cows, all of which were Jerseys. Considerable data were available on the blood serum citric acid levels on the day before, the day of and the day following parturition. When these data were submitted to an analysis of variance, there was no significant variation in blood serum citric acid levels between breeds or between prepartum and non-prepartum milked cows.

It is evident from table 1 that there is some relation between calcium and citric acid levels of the blood serum in both normal and milk fever cows. Increases or decreases in serum calcium are accompanied by concurrent increases or decreases in serum citric acid. A statistical analysis of the data based on 231 comparisons in cows calving normally showed a correlation of +0.40 between calcium and citric acid levels of the blood serum. In milk fever cows the correlation based on 76 comparisons was +0.46. In both normal and milk fever cows these figures represent highly significant correlations between calcium and citric acid levels of the blood serum.

Data were available on blood serum citric acid levels for 17 cows both 3 and 1 days prepartum. An analysis of variance of these data did not indicate any significant differences in serum citric acid levels between 3 and 1 days prepartum in either the normally calving or the milk fever cows.

Table 1 indicates a drop in mean level of serum citric acid in both normally calving and milk fever cows at the time of parturition. An analysis of variance involving blood serum citric acid levels on 33 cows 1 day prepartum, the day of parturition, and 1 day postpartum was made. For convenience of discussion, +1 will be used to indicate one day prepartum; 0, the day of parturition; and -1, one day postpartum. The apparent drop in serum citric acid between +1 and -1 was found to be highly significant for both milk fever and normally calving cows. There was a greater drop in blood serum citric acid in milk fever than in normal cows. The difference between normally calving and milk fever cows in this respect was highly significant.

Based on 3-day totals (+1, 0, -1) there were no significant differences in citric acid levels of the blood serum between milk fever and normally calving cows. This fact, plus the greater drop (highly significant) in blood serum citric acid levels in milk fever cows over the same period of time, indicates indirectly that serum citric acid levels were significantly higher in milk fever cows than in normally calving cows 1 day prepartum. This difference between normally calving and milk fever cows is borne out by the data presented in table 1. On day +1 the cows which subsequently developed milk fever had a mean level of 8.63 mg. per cent citric acid in their blood serum as compared to 6.24 mg. per cent for the normally calving cows. By day -1 blood serum citric acid in milk fever cows had declined to 2.20 mg. per cent as compared to 3.73 mg. per cent in normally calving cows.

Data on the citric acid levels of the blood serum for the day of parturition and the first 3 days subsequent to parturition (0, -1, -2, -3) were available on 42 cows (264 analyses). An analysis of variance was made on these data. The following facts became evident as a result of this analysis: (a) Citric acid values increased more slowly following parturition in cows developing milk fever than in normally calving cows (highly significant). (b) Citric acid levels of the blood serum were lower for the period studied (0, -1, -2, -3) in milk fever cows than in normally calving cows (highly significant). (c) The citric acid values for the day of parturition as compared to 3 days postpartum were significantly different for all cows; in the normally calving cows, citric acid values were higher 3 days postpartum than at parturition, but in the milk fever cows the reverse situation was true.

The length of time during which serum citric acid values persisted at a low level following calving in the milk fever group can be at least partially explained by relapses which occurred in several of the milk fever cows as late as 3 days postpartum. The relapses which occurred were consistently associated with low serum citric acid values. If more data were available, it would be interesting to study the speed with which serum citric acid levels recover following the last attack of milk fever.

Both normal and milk fever cows exhibit somewhat higher citric acid levels on the third day prepartum than on the third day postpartum. The difference is much more marked in milk fever than in normal cows.

Considering the blood serum citric acid picture in its entirety, a drop in serum citric acid has been demonstrated to occur at parturition in both normal and milk fever cows. This drop closely parallels the drop in blood serum calcium which has been demonstrated many times by numerous workers. The prepartum levels of serum citric acid are greater in milk fever than in normally calving cows, and the drop is also of greater magnitude and to lower levels in the former. Low levels of serum citric acid persist for a longer time in milk fever than in normal cows.

The correlation between citric acid and calcium levels of the blood serum at the time of parturition having been established, it is difficult to say which of the two changes that occur in milk fever is the more basic. No likely explanation is advanced herewith. The demonstration by Shorr *et al.* (11, 12) that both estrogens and androgens play a role in calcium and citric acid metabolism makes possible the speculation that they are responsible in some way for the changes occurring in citric acid and calcium levels of the blood at the time of parturition, since levels of the steroidal hormones are undergoing marked changes during this period.

#### SUMMARY

Blood serum citric acid and calcium analyses were run on twenty-two Jersey, six Guernsey and four Holstein cows from 10 days prepartum to 10 days postpartum. There were no significant differences in blood serum citric acid between breeds or between prepartum- and non-prepartum-milked cows. There was a

highly significant correlation between blood serum citric acid and calcium over this period of time. Serum citric acid levels did not show any appreciable change between 3 and 1 days prepartum, but there was a definite drop in both milk fever and normally calving cows between 1 day prepartum and 1 day postpartum. This drop was of greater magnitude in milk fever than in normal cows.

Following parturition, serum citric acid levels increased in normally calving cows and decreased in cows which developed milk fever. Part of this difference in behavior can be attributed to relapses which occurred in several of the cows with milk fever.

#### ACKNOWLEDGMENT

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It is our pleasure, after sixteen years, to welcome you back this summer to the campus of Cornell University in the heart of our great dairy state of New York. Our grounds, our buildings, our staff are being readied for you as you filter into our midst from all parts of our country, Canada and many foreign lands. The days of June 20, 21 and 22 with you as our guests and we as your host, we anticipate with joyously warm feelings and with pride denoting no small amount of satisfaction that you have chosen Cornell as the place for your 1950 annual meeting.

The priceless tradition of our University founded by Ezra Cornell is that of freedom and public service which has been spelled out in our colleges from its very opening. The love of truth and the disinterested pursuit of truth wherever it leads, is what we seek. Your American Dairy Science Association has similar objectives as you approach the multitude of problems in the fields of dairy production, manufacturing, marketing and in your resident and extension teaching. These fundamental freedoms which the living spirit of Cornell exemplifies, you definitely promulgate and adhere to. No more fitting place could have been located, we hope, for your annual meeting. Our traditions are mutual.

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Sincerely yours,  
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### PAPERS FOR THE 1950 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association will be held June 20 to 22, 1950, at Cornell University, Ithaca, N. Y. All members who are planning to present papers should submit the title of their paper accompanied by *two* copies of an abstract of not more than 200 words not later than March 15 to the chairman of the program committee of their respective section. The committee chairmen are as follows:

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Because of the difficulties encountered in showing slides, it is recommended that each speaker distribute mimeographed copies of his data, together with a brief summary of his paper.

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## THE CURVILINEARITY OF HERITABILITY OF BUTTERFAT PRODUCTION<sup>1</sup>

JOHN P. BEARDSLEY,<sup>2</sup> R. W. BRATTON, AND G. W. SALISBURY<sup>3</sup>

*Laboratory of Animal Breeding and Artificial Insemination,  
Department of Animal Husbandry, Cornell University, Ithaca, New York*

The efficiency of selection of breeding animals is seriously limited because the phenotype of an economic quantitative characteristic is the result not only of genetic but also of environmental influences. The relative contribution of genetic and environmental influences is, therefore, information of significance.

Heritability is used as the measure of the portion of phenotypic variability which can be attributed to additive genetic deviations. The remaining portion of variability may be ascribed to environmental deviations and to any deviations resulting from dominance, over-dominance, epistasis, and non-linear interactions of heredity and environment.

A number of studies have shown that the heritability of most milk and butterfat records is of the order of 20 to 30 per cent (4, 5, 6, 7, 8). Carneiro and Lush (2) in a preliminary observation on Brazilian cattle of low average production and unusual genetic heterogeneity found heritability to be about 50 per cent. Studies with other animals have raised the question of whether or not heritability is a constant. Wright (12), studying the quantitative inheritance of piebald spotting in guinea pigs found that homozygosity, produced by inbreeding, reduced the portion of variation in spotting which was inherited. Later, Hetzer, *et al.* (3) found that heritability of type in swine was greater in crosses between divergent types than it was in matings within a type.

In the case of butterfat production of dairy cattle, one could speculate that either or both of the following situations would result in decreased estimates of heritability as production levels increased: (a) If high production is the result of homozygosity, lower genetic variance and heritability may be found at higher levels of production. (b) If high production is a consequence of dominance, over-dominance or epistasis, additive genetic variance and heritability may be smaller at higher levels.

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<sup>1</sup> The data published here are taken from a thesis presented by the senior author to the Faculty of the Graduate School, Cornell, University in partial fulfillment of the requirements for the Degree of Master of Science, June, 1948.

<sup>2</sup> Now Director of Research, The American Jersey Cattle Club, Columbus, Ohio.

<sup>3</sup> Now in the Department of Dairy Science, University of Illinois, Urbana.

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The present paper gives the results of a study designed to test the assumption that heritability varies with level of butterfat production.

#### METHOD OF INVESTIGATION

The data were taken from "Proved-Sire Records" as issued by the Division of Dairy Herd Improvement Investigations, Bureau of Dairy Industry, U. S. Department of Agriculture. The present study is incidental to another and the data meet the specifications of the latter; therefore, each sire has a minimum of five daughter-dam comparisons in each of two or more herds. This fact could make the data atypical of DHIA data; however, the authors fail to see how this could influence greatly the problem under investigation. All individual butterfat records are the average, mature equivalent records for twice-a-day milking, 305-day-lactation periods. Table 1 presents a summary of the data used.

TABLE 1  
*Summary of the data from "Proved-Sire Records" used for the study of heritability*

Breed	No. of sires	No. of herds	No. of daughter-dam comparisons
Holstein	120	271	2336
Jersey	32	66	544
Guernsey	24	53	427
Total	176	390	3307

The pooling of the variances of three breeds was justified first. The test of the homogeneity of variances, developed by Bartlett, was employed as described by Snedecor (10).

A multiple regression of daughters' butterfat production (Y) on dams' butterfat production (X) within breeds, sires and herds was computed for the purpose of estimating the curve of heritability. A fitted curve must be interpreted with caution, for it attempts to describe the situation only within the range of data studied; consequently, the extremes of the curve can be very misleading. The derivation of a curvilinear regression, however, does permit a statistical test of significance of curvilinearity. A modified polynomial curve was used to describe the regression.

A single coefficient cannot describe a curvilinear regression; therefore, heritability cannot be estimated by doubling a single regression coefficient as is the usual technique (4). Instead, it is necessary to estimate average linear regression coefficients at varying levels of butterfat production. These can be doubled to obtain indications of the heritability at various productive levels. Estimated daughters' production (Y) was computed from the multiple regression equation with dams' production (X) set at 100-lb. intervals. By setting X at 100-lb. intervals, the change in estimated Y over a 100-lb. interval of X becomes an estimate of the linear regression of Y on X at the midpoint of the interval considered.

#### RESULTS

Bartlett's test of the homogeneity of the three breed variances yielded a chi-

square value of 1.529 which falls between the 0.50 and 0.30 levels of probability. The statistical probabilities of real differences existing between the variances are low enough that the pooling of the variances was justified.

The equation of curvilinear regression of daughter's butterfat production on dam's butterfat production within breeds, within sires, and within herds, was found to be as follows:

$$Y = 149.6 - 0.092 X - 2.553 \sqrt{X} + 47.03^3 \sqrt{X}$$

$$R^2 = 0.018 \quad R = 0.134$$

The linear regression of daughter's butterfat production on dam's butterfat production within breeds, within sires and within herds ( $b_{yx}$ ) was found to be 0.137. The corresponding correlation coefficient is 0.127.

The test of significance of the curvilinearity of regression revealed that curvilinear regression does not differ significantly from linear regression. In view of the fact that curvilinearity approaches statistical significance and that the curve does fit the data more closely, calculations of curvilinear heritability were felt to be justified. However, it must be remembered that curvilinearity has not been definitely established and that more work is needed to prove or disprove its reality. A summary of this test is presented in table 2.

TABLE 2

*Test of significance of curvilinearity of regression of daughters' butterfat production (Y) on dams' butterfat production (X)*

Source of Variation	d.f.	S.S.E.E. (1-r <sup>2</sup> )Sy <sup>2</sup>	M.S.
Deviations from linear regression	2916	10,796,791.76	
Deviations from curvilinear regression	2914	10,777,796.64	3,698.63
Curvilinearity of regression	2	18,955.12	9,497.56
F = 2.568 (not significant: 5% = 3.00)			

An estimate of average heritability of butter fat production may be obtained by doubling  $b_{yx}$ . It was found to be 27.4 per cent. The summary and results of the computations of curvilinear heritability of butterfat production are presented in table 3.

TABLE 3

*The computation of the curvilinearity of heritability of butterfat production*

Dam's production ( $\bar{X}$ )	200	300	400	500	600
Estimated $\bar{Y}$ . . . . .	370.1	392.6	408.3	419.8	428.3
Increase of $\hat{Y}$ over $\hat{Y}$ of next lower level of $\bar{X}$		22.5	15.7	11.5	8.5
2 (increase of $\hat{Y}$ ) approximate av.% heritability		45.0	31.4	23.0	17.0
Av. level of $\bar{X}$ for the estimated % heritability		250	350	450	550

#### DISCUSSION

A comparison of the measurements of the curvilinear and linear relationships between daughters' butterfat production and dams' butterfat production with-



in breeds, within sires and within herds reveals that the multiple correlation coefficient ( $R = 0.134$ ) is higher than the simple correlation coefficient ( $r = 0.127$ ). The difference between linear and curvilinear regression, however, did not quite reach statistical significance at the 5 per cent level of probability. This is not evidence that the true regression is necessarily linear, for the curvilinear regression does fit the data more closely and, consequently, is the more probable one.

The genetic reasons for the observed trend of heritability as found in this study are not readily apparent. Two or more different effects may be at work. One possible explanation is that high production may be the result of homozygosity. In this case, as in that of Wright with spotting in guinea pigs (12), such genetic homozygosity would result in a smaller proportion of the observed variability of the characteristic being transmitted from generation to generation as the homozygosity increased. Results of inbreeding studies (1, 9, 11, 13) do not lend much support to this explanation. There is the possibility, however, that continued selection for high production may lead to homozygosity in some instances without the detrimental effects observed in most inbreeding experiments.

A second possible explanation, and there probably are others, is that high production may represent in many instances non-additive genetic deviations in addition to additive genetic influences. These non-additive genetic variations could be attributed to dominance, over-dominance, epistasis and gene-environmental actions. For all practical purposes, such effects are lumped with environmental deviations in the present methods of study and are not distinguishable from them. Also, from the standpoint of selection on the basis of individual performance these effects are non-transmissible.

It is impossible to determine which of these explanations is more probable or more important, though the second seems more likely. If the second is more important, the selection of breeding animals should be directed toward those individuals or families showing the desired phenotype in the progeny, and less emphasis should be placed on the performance of individuals.

#### SUMMARY

A statistical study of butterfat records of the progeny and mates of 176 proved sires of the Guernsey, Holstein-Friesian, and Jersey breeds has been made. Each bull was represented by at least five daughter-dam comparisons in each of two or more herds.

Curvilinear regression of daughter on dam within breeds, within sires and within herds accounted for a larger portion of the daughter variance than did linear regression. The difference, however, was not quite large enough to be statistically significant.

The heritability of butterfat yield calculated by doubling the linear regression of daughter on dam within breeds, within sires and within herds was 27.4 per cent. Estimates of heritability on the basis of curvilinear regression gave values decreasing with increased butterfat yield. The pattern described by these estimates appears to hold a valid relation to the problems faced by breeders

of dairy cattle and to the results of some experimental studies on similar problems.

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## EXTRACTION AND ISOLATION OF GAMMA GLOBULIN FROM THE BOVINE THYMUS GLAND<sup>1</sup>

SYED KAMAL AND C. W. TURNER

*Department of Dairy Husbandry  
University of Missouri, Columbia*

The high mortality rate in young animals is a serious economic problem in the livestock industry in general and the dairy industry in particular. In addition to the use of improved methods of management, feeding and breeding, it is important to consider the inherent deficiency of the newborn animal as regards the immune globulin content of its blood. Nature overcomes this deficiency by providing the newborn animal with antibody-rich, immune lactoglobulin in its dam's colostrum. Furthermore, the immunity imparted through colostrum feeding is of a transitory nature and the young animal is more susceptible to diseases until such time as its own gamma globulin content of the blood attains a normal level. The possibility of overcoming this inherent deficiency by supplying the newborn animal with immune globulin in addition to colostrum feeding, either from the blood or from other tissues, seems feasible.

It was proved over half a century ago that specific antibodies to nursing mice were transmitted in the colostrum (6). Also, it has been demonstrated that placental transmission plays no important role in ruminants and that the transmission of immunity is mainly through colostrum feeding in newborn animals (8, 16, 18).

The blood serum of newborn calves is deficient in globulin and such animals, if not allowed to suckle, are unusually susceptible to colon bacillus septicemia (23). Furthermore, it has been shown by means of electrophoretic studies that the blood of a newborn calf lacks immune globulin and that an immediate increase in the gamma globulin content of the blood of newborn calves occurs following the ingestion of colostrum during the first 24 hr. (9, 12). The fact that in young animals the serum protein values are normally below adult values has a possible bearing upon the increased susceptibility of young calves to many infectious agents (4).

Numerous investigators have demonstrated that the lymph glands are sites of antibody formation (5, 13, 19). Many investigators also, have shown the similarity and inseparable nature of antibodies from immune globulin with which they are invariably associated (2, 4, 5, 22, 24).

Recent endocrine work indicated that lymph glands such as the thymus of small animals contained gamma globulin (25). The gamma globulin was thought to be released and regulated by the adrenal cortical hormones. It was thought that these tissues might serve as a rich source of gamma or immune globulin. Therefore, attempts were made to extract gamma globulin from the bovine thymi.

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## EXPERIMENTAL MATERIAL AND METHODS

Bovine thymus glands were obtained from the packing house immediately after slaughtering the animals. The glands were carefully dissected, fascia and fat being removed. These then were weighed into 50 g. lots, wrapped in butter paper and kept at  $-15^{\circ}\text{C}$ .

Acetone-dried and ether-defatted thymus tissue used in these experiments was prepared mainly according to the method described by Bergman and Turner (1) for the preparation of dehydrated pituitary powder except that the procedure was carried out at  $-5^{\circ}\text{C}$ . to avoid denaturation of the thymus tissue proteins.

Calf thymus desiccated at  $40^{\circ}\text{C}$ . used in these experiments was obtained from the Viobin Corporation, Monticello, Ill.

The procedure followed for the extraction of salt soluble proteins from the thymus glands was according to that described by Luck (17) for the extraction of proteins from liver. One molar NaCl solution was used to dissolve thymocytes and pH was adjusted to 5.0 with 0.05 *M* acetic acid to precipitate nucleoproteins, as recommended by Mirsky and Hans (20). Thymocytes also were extracted with a 20 per cent solution of NaCl according to the method described by White and Dougherty (35).

Mincing of the thymus tissue and stirring for extraction of the minced tissue was carried out in the cold, whereas centrifugation for the recovery of supernatant from the saline tissue extract was carried out at 3500 R.P.M. for one hr. at room temperature in the absence of a refrigerated centrifuge.

The general scheme followed for the separation of gamma globulin from the saline thymus extracts was according to the ethanol precipitation procedure described by Hess and Deutsch (11) for the serum of normal cows. The precipitation of gamma globulin from the saline thymus extracts also was attempted with ammonium sulfate, according to the procedure described by Cohn and coworkers (3) for the normal serum of the horse.

## RESULTS

Only 60 mg. of acetone-dried gamma globulin was obtained from a 20 per cent saline extract of 50 g. of frozen bovine thymus by precipitating it at 34 per cent saturation with ammonium sulfate at pH 6.0. The ethanol precipitation procedure of Hess and Deutsch was ineffective in recovering gamma globulin.

## DISCUSSION

It was not possible to extract and precipitate an appreciable quantity of gamma globulin from bovine thymus with either ethanol or salting-out procedures usually employed for blood. The negative results in these experiments may be explained as follows: First, there may be species differences. The hypothesis advanced by White and Dougherty (25) that the lymphoid tissues in small animals (rabbits and mice) are store houses of gamma globulin may not apply to large animals. Second, other investigators have failed to duplicate the results of White and Dougherty (25) in small animals. It has been shown that the level of serum albumin in the rat is under the control of the adrenal cortex (14). Also,

it has not been found possible to obtain evidence that adrenotrophic hormone causes a significant elevation in the concentration of the globulin fractions of the plasma or lymph of rats treated with adrenotrophic hormone (15). Furthermore, adrenal cortical activity in the rat is not essential for the fabrication or release of antibodies and gamma globulin (11). It also has been shown that adrenalectomized rabbits with hypertrophy of the lymphoid tissues produce antibodies far in excess of that produced by intact animals (21).

A very recent comparative electrophoretic and ultracentrifuge study has been made of the saline extracts of lymphocytes from popliteal (regional) lymph nodes of the hind feet of rabbits infected with killed dysentery organisms (10). The components with higher electrophoretic mobilities were increased after the injection of antigen, whereas the gamma globulin was not increased significantly.

The presence of a component of the same electrophoretic mobility as the gamma globulin of the blood in the lymphoid cells, as reported by White and Dougherty (25), also has been demonstrated by other investigators (10, 13). Therefore, there can be no question as to the presence of gamma globulin in the lymphoid cells, but these studies indicate that the amounts present in bovine thymi are not present in sufficient amounts to be extracted and precipitated by the procedures employed.

#### SUMMARY

1. Fresh bovine thymus glands, acetone-dried and ether-defatted thymus tissue, and desiccated calf thymus tissue were extracted with 1 *M* saline solution. No gamma globulin could be precipitated from these extracts either by adjusting the pH to 7.7 and ethanol concentration to 18 per cent by volume at  $-10^{\circ}\text{C}$ ., or by ammonium sulphate at 34 per cent saturation at pH 6.

2. Fresh bovine thymus glands were washed three times with physiological saline and lysed with one volume of distilled water and then extracted with one volume of 20 per cent saline. The saline extract yielded 60 mg. of acetone-dried gamma globulin on 34 per cent saturation with ammonium sulphate at pH 6.

3. It is concluded that the bovine thymus, though containing small amounts of gamma globulin, cannot be used as a rich source of immune globulin.

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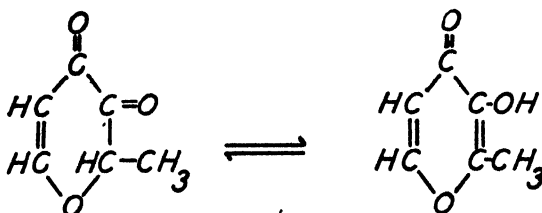
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# THE ISOLATION OF MALTOL<sup>1</sup> FROM HEATED SKIM MILK<sup>2</sup>

STUART PATTON

*Department of Dairy Husbandry  
The Pennsylvania Agricultural Experiment Station  
State College, Pennsylvania*

In an effort to clarify the nature of heat-induced chemical changes in milk and certain other dairy products, it has been the object of research in these laboratories to isolate and identify compounds produced in milk by heat. Previous studies have demonstrated the formation of furfuryl alcohol in skim milk as a result of heating (7). This paper reports the isolation and identification of maltol from heated skim milk and presents certain observations concerning its origin in this medium. The molecular constitution of maltol, which has been proven by Peratoner and Tamburello (8), is as follows:



## EXPERIMENTAL

During the course of previous experiments (7), it was observed that distillation residues from the ether extract of heated skim milk gave rise to crystals on standing for a few days in the cold. Further, these crystals upon heating in vacuo would sublime from the residue. When this sublimed material was recrystallized several times from toluene, a pure crystalline compound (m.p. 159° C.) was obtained. This compound even in very high dilution gave an intense purple color with ferric chloride reagent. These preliminary observations served to characterize the compound for further research purposes.

Yields of the compound in these first experiments were extremely small (approximately 50 mg. from 20 gal. of heated skim milk). In order to improve the yield, several modifications of procedure were tried and the following one adopted for preparation of the compound: Five liters of condensed skim milk (29 per cent total solids) was autoclaved for 2.5 hr. at 127° C. The autoclaved milk was allowed to cool to room temperature. The whey was poured off and extracted three times with an equal volume of 80 per cent ether and 20 per cent

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<sup>1</sup> 3-hydroxy-2-methyl-pyrone (4)

<sup>2</sup> Authorized for publication as paper no. 1548 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

methanol solvent mixture. The curd was broken up and washed three times using the above solvent. Final extraction of the curd was accomplished by allowing it to stand in the solvent overnight. The extracts of the whey and curd were combined and concentrated by evaporating the solvent on a steam bath. When the extract had been reduced to a volume of approximately 200 ml., it was dried over anhydrous sodium sulfate and the remnants of the solvent then removed on the steam bath. The extract residue was taken up in 50 ml. of hot toluene and the clear toluene layer decanted. On cooling, the toluene solution gave rise to a crude brown crystalline mass which on several recrystallizations from a mixture of toluene and ligroin yielded approximately 100 mg. of compound, m.p. 159° C. Six such experiments were required in order to obtain a sufficient quantity of the compound for identification purposes.

*Characteristics of the compound from heated skim milk.* In addition to the m.p. of 159° C., the property of sublimation, and the positive ferric chloride test, the following characteristics were observed for the compound: soluble in chloroform and alcohol; difficultly soluble in water, ether, benzene, and toluene; insoluble in petroleum ether and carbon tetrachloride; acid to litmus but ether-extractable and steam-distillable from 10 per cent sodium bicarbonate solution; did not give a satisfactory titration for neutralization equivalent; reacted with iodine, potassium iodide and sodium hydroxide to give iodoform; reduced neutral potassium permanganate, alkaline silver nitrate and Fehling's solution. The compound gave negative results with 2,4-dinitrophenylhydrazine, aniline and acetic acid, bromine in carbon tetrachloride and  $\alpha$ -naphthol and concentrated sulfuric acid reagents.

Carbon and hydrogen analyses of the compound purified by several recrystallizations followed by sublimation gave 56.79 per cent carbon and 4.89 per cent hydrogen. Molecular weight of the compound (camphor melting point depression method) was determined as 128. These data calculated well to a molecular formula of  $C_6H_6O_3$  for which carbon equals 57.14 per cent, hydrogen equals 4.80 per cent and molecular weight equals 126.

A search of the literature revealed that the properties of the compound isolated from heated skim milk were in good agreement with those compiled for maltol (1, 6).

*Confirmation of identity.* Of the three derivatives of maltol which are reported in the literature, namely, the methyl ether, benzoate ester and phenyl urethane, only the latter two are solids and, therefore, suitable for preparation in small quantities. Accordingly, the benzoate ester was prepared by the method of Feuerstein (4) and found to melt at 114–115° C. (given in the literature 114–115° C.). The phenyl urethane, prepared according to the method of Peratoner and Tamburello (8) melted at 151–152° C. (given in the literature 149–150° C.). As a final check, a mixed melting point was performed with the compound isolated from heated skim milk and a known sample of maltol donated by J. R. Schenck of the Abbott Research Laboratories. This sample of maltol was prepared from wood tar by the Cliffs Dow Chemical Co. (5). These two samples melted sharply at 159° C., both alone and mixed.



*Origin of maltol in heated skim milk.* In an attempt to determine the origin of maltol in heated skim milk two series of simplified systems were prepared. The first of these consisted of 7.5 per cent of purified casein (Arthur H. Thomas) together with 15 per cent of lactose, glucose or galactose (anhydrous, C.P. grade), respectively, made up in aqueous solution. Samples (750 g.) of the sugar-casein systems were autoclaved for 2.5 hr. at 127° C., cooled and extracted with ether-methanol mixture as previously described. The solvent-free extract residues were tested with ferric chloride reagent (3 per cent aqueous). That from lactose was positive while those of glucose and galactose were negative.

These findings suggested that the formation of maltol involved a lactose-casein interaction in which the intact lactose molecule was required. Since it is known that maltol can be produced by the pyrolysis of cellulose or starch alone (3), a catalytic role might be suggested for casein in this instance. To investigate this point further, a second series of simplified systems were prepared in which 3 per cent of glycine was substituted for the casein. The pH values of these systems were  $7.0 \pm 0.2$ . One kg. samples of the sugar-glycine systems were autoclaved for 6 hr. at 127° C. and cooled to room temperature. A slightly different method was used to concentrate the maltol. The autoclaved samples were saturated with sodium chloride and then steam distilled. The sodium chloride was found to facilitate the steam distillation of maltol considerably. The steam distillate from the lactose-glycine system gave a strong purple ferric chloride reaction. The reaction became gradually weaker and was negative after 5 l. of distillate had been collected. This distillate was extracted with an equal volume of chloroform which treatment effectively extracted the maltol from the distillate, as indicated by the ferric chloride test. The chloroform was evaporated off on a steam bath. The residue containing a small amount of chloroform was cooled in a refrigerator for several hours after which time crystals had appeared. Further crystallization was induced by the addition of a small quantity of petroleum ether. The crystals were filtered off, washed with cold ether, dried and weighed. A yield of 55 mg. of maltol, m.p. 158–159° C., was obtained from the lactose-glycine system. The steam distillates from the glucose and galactose systems, as well as that of a control lactose sample, were negative to the ferric chloride test. Since even a few milligrams of maltol dissolved in several liters of water will give a positive ferric chloride test, it seemed safe to conclude that the latter distillates contained no maltol.

#### DISCUSSION

Maltol is a rather obscure and somewhat unusual compound. It occurs in the bark of the larch tree (10) and in the needles of the silver fir tree (4). It also is formed during the roasting of malt (2), the dry distillation of cellulose and starch and the carbonization of wood (3, 5). Maltol also is formed by the degradation of streptomycin with alkali (9).

The isolation of maltol from heated skim milk may be of interest for several reasons. It seems clear from these studies that the compound has its origin in lactose and thus maltol represents one of the possible avenues of lactose degradation in heated milk. Further, although maltol can be formed from polysaccha-

rides, lactose, insofar as is known, is the first disaccharide demonstrated to serve as an origin. This may throw some light on the fundamental nature of maltol formation from carbohydrates. The fact that maltol could not be produced from either glucose or galactose in these experiments emphasizes the importance of the intact lactose molecule.

Based on the capacity of casein and the amino acid glycine to convert small quantities of lactose to maltol, a catalytic role may be indicated for the milk proteins in the formation of the compound in heated skim milk.

It appears unlikely that maltol occurs to any extent in heat processed dairy products unless they have undergone considerable browning as a result of rigorous heat treatment, prolonged storage or both. This matter will bear further investigation. It is known from previous experiments (7) that maltol is formed in skim milk heated for 90 min. at 127° C. How much this heat treatment may be reduced with detectable quantities of maltol being produced is not known. Steam distillation of the medium in question coupled with the ferric chloride test would be a very sensitive method of detection. It also is possible, as suggested by Schenck and Spielman (9), that the stable purple color, produced in the ferric chloride test, might lend itself well to colorimetric measurement, thus enabling quantitative measurement of maltol.

Samples of maltol from carbonized wood, streptomycin and heated milk all have a pleasant sweetish odor reminiscent of burnt sugar. This odor would appear to be characteristic of the compound, rather than one of contamination. In the quantities found in heated milk, maltol probably contributes little in the way of flavor or odor.

#### SUMMARY AND CONCLUSIONS

Maltol is one of the compounds formed in skim milk as a result of prolonged heat treatment at high temperatures. The amount of maltol produced in milk appears to increase with increasing concentration and heat treatment of the milk. The formation of this compound is correlated with browning of the milk and more specifically, depends upon the interaction of the milk proteins upon lactose. These experiments indicate that the complete lactose molecule is required, since maltol could not be produced from either glucose or galactose. A purified sample of casein and the amino acid glycine both were found capable of converting small quantities of lactose to maltol. A catalytic role seems possible for the proteins in the reactions by which maltol is formed in heated milk.

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construed as necessarily reflecting the views or endorsement of the Department of the Army.

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# OCCURRENCE OF MICROCOCCI IN CHEDDAR CHEESE MADE FROM RAW AND FROM PASTEURIZED MILK<sup>1</sup>

JOHN A. ALFORD<sup>2</sup> AND W. C. FRAZIER

*Department of Agricultural Bacteriology, University of Wisconsin, Madison*

It long has been recognized that a desirable flavor develops more rapidly in cheddar cheese made from raw milk than in cheese from pasteurized milk. However, since more cheese of an inferior grade results from the use of raw milk, many cheesemakers sacrifice the added flavor development and use pasteurized milk to obtain cheese of a more uniform quality.

There have been numerous investigations of the bacterial flora of cheddar cheese made from raw milk in an effort to relate the organisms found to the ripening of the cheese. Hastings *et al.* (4) were among the first to show that streptococci were the predominant organisms in raw milk cheese during early ripening and were slowly supplanted by the lactobacilli. Other investigators (9) have found a similar development of streptococci and lactobacilli but none has been able to correlate the numbers of these organisms with the rate of ripening or the intensity of flavor development. Tittler *et al.* (9) have shown that there are few lactobacilli present in cheese made from pasteurized milk.

Evans *et al.* (2) found micrococci in raw milk cheese but noted no correlation between the total numbers and flavor development. Rogers (8) suggested that the micrococci were present in sufficient numbers to be important in cheese ripening. Micrococci have been known for many years to be present in milk drawn aseptically from the udder (3). The chief argument against consideration of the micrococci as a factor in cheese ripening has been that they occur in such small numbers and disappear so rapidly that they would be of little or no importance. Rogers (8) stated that in any study of the bacteriology of cheddar cheese ripening it would be necessary to find cultures that would dominate the bacterial population. He believed it necessary to distinguish between those bacteria growing in the milk and those that grow in cheese in the curing room. Orla-Jensen (6) did not consider living bacteria essential to the ripening process. He believed that the enzymes released upon death and autolysis of the bacterial cells were most important.

This investigation was made to determine if any of the organisms found in raw milk cheese, other than the lactic acid bacteria, occurred in sufficient numbers to be of importance in the development of flavor in the cheese.

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<sup>2</sup> Present Address: Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge.

## EXPERIMENTAL PROCEDURE

The milk used for the comparison of the flora of raw milk cheese and pasteurized milk cheese was from the regular supply of the Dairy Industry Department of the University of Wisconsin. One half of the milk was pasteurized at 62.5° C. for 30 min., while the remainder served as the raw milk control. Cheese was manufactured in 450-lb. vats according to the usual methods (7).

The predominant bacteria in young cheese are the streptococci that originated from the starter. Other organisms are present in much smaller numbers and will be overgrown on any medium that permits normal growth of the lactics. It was necessary, therefore, to use a medium that would not support good growth of the lactics but would give well developed colonies of many non-lactic types. One per cent tryptone agar (tryptone, 10 g.; agar, 15 g.; water, 1000 ml.) was found to give the desired selectivity. Appropriate dilutions of 1-ml. samples of milk and 1-g. samples of curd and cheese were plated in the tryptone agar. Plates were incubated at 30° C. for 4 to 5 days before they were counted and colonies were isolated from them.

Petri dishes showing well isolated colonies were selected from each sample. All of the colonies from a representative section of the plates were picked and inoculated into semi-solid tryptone agar (0.3% agar). After incubation for 24 to 48 hr. at 30° C., the cultures were streaked on tryptone agar plates and these plates incubated at 30° C. for 48 hr. An isolated colony from each streak was picked into tryptone agar. This purification procedure was necessary to eliminate the microscopic colonies of lactic acid bacteria that often were carried over from the original tryptone agar plates. The cultures were divided into groups on the basis of morphology, pigmentation, action on litmus milk, proteolysis in milk agar, lipolysis in tributyrin and butterfat agar, growth in 6.5 per cent sodium chloride and growth at pH 5.2. These characteristics were selected rather than the usual ones used in differentiating bacterial species because they would give a better indication of the possible importance of the isolated organisms in cheese ripening.

To indicate further which organisms might be important in the ripening of cheese, sterilized milk was inoculated with 0.8 per cent of a *Streptococcus lactis* culture in milk and a similar number of micrococci from a selected micrococcus culture. Counts were made on these mixtures immediately and after 2 days of incubation at 30° C. to determine whether the micrococci were capable of multiplication in the presence of *S. lactis*.

The intensity of cheddar cheese flavor in the cheeses was recorded by use of a scale running from zero to four. The flavor intensities presented in the graphs are the averages of the intensities assigned by the different judges.

## RESULTS

Sixteen pairs of cheeses were made to compare raw milk cheese with that made from pasteurized milk in regard to non-lactic flora and the rate of flavor development. Tryptone agar counts were made on the milk after addition of the starter, on the cheese at pressing and periodically during the ripening. All cheeses were

ripened at 5 to 7° C. The results obtained on paired raw and pasteurized milk cheeses are given in figure 1.

It is apparent that organisms other than lactic acid bacteria were present in raw milk cheeses in numbers ranging from 600,000 to 10,000,000 per g. by the time the cheeses were 1 day old. The non-lactics in cheese made from the same milk that had been pasteurized rarely exceeded 10,000 per g. Although the maximum number of non-lactics in raw milk cheese varied considerably, the counts on cheeses made from pasteurized milk were very close to one another. This suggests that the organisms that survived pasteurization multiplied at about the same rate, whereas those in raw milk cheese varied widely in the

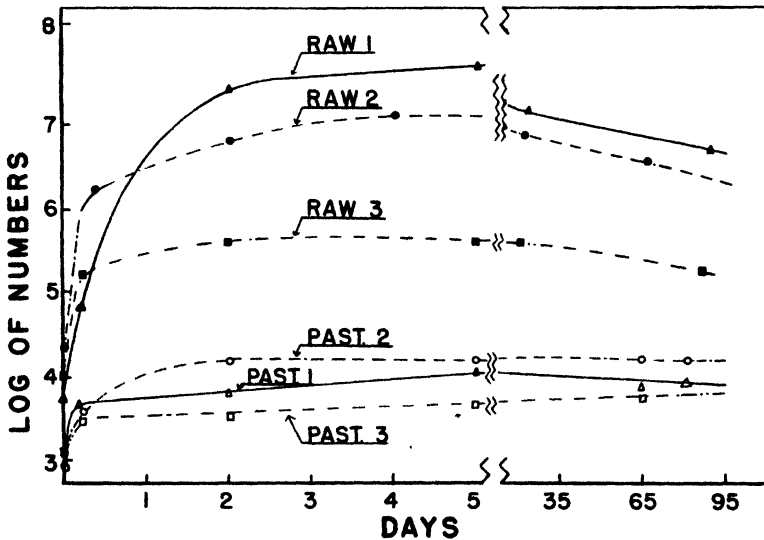


FIG. 1. Comparison of numbers of non-lactic bacteria in cheese made from raw and from pasteurized milk.

amount of their growth. There is a five- to eight-fold increase in the number of cells in curd as a result of the removal of the whey, and most of the apparent multiplication of non-lactics in the pasteurized milk cheese can be accounted for on the basis of this concentration. However, the number of non-lactics in the cheese from raw milk greatly exceeds the count that can be attributed to this cause. Most of the increase in count occurred during manufacture and while the cheese was still in the press.

Figure 2 contains a comparison of the flavor development in cheese made from raw and from pasteurized milk. This method of presentation of data is used because the rate as well as the amount of flavor development can be presented. The development of flavor was more rapid during the first few months in the cheese made from raw milk than in the companion cheese for which the milk had been pasteurized. Further increases in flavor, however, proceeded at about the same rate in the raw and in the pasteurized milk cheese. For example, a flavor intensity of 2 developed in 5 mo. in raw milk cheese no. 2, whereas a

flavor intensity of 1 had developed in that time in pasteurized milk cheese no. 2. At the end of the next 3-mo. period, however, both cheeses had increased in flavor intensity to 3. Similar relationships are evident in the other paired lots, al-

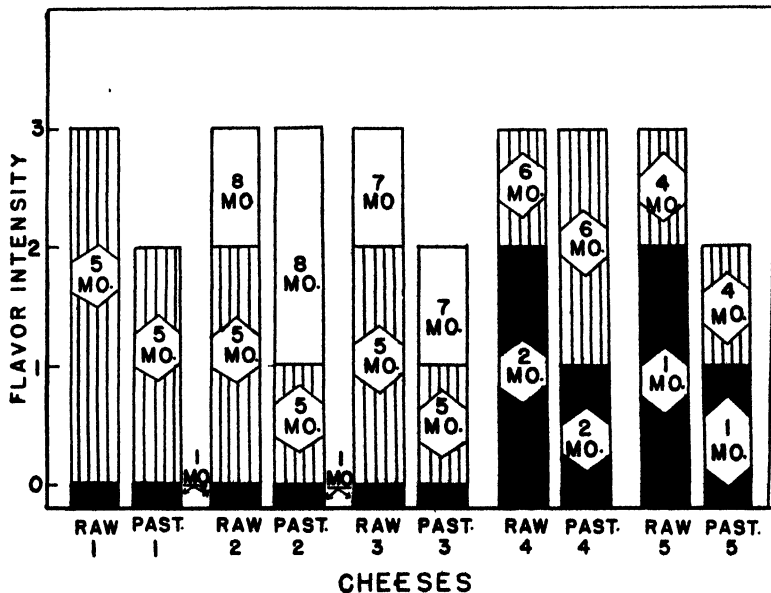


FIG. 2. Comparison of the rates of flavor development in cheese made from raw and from pasteurized milk.

though in some of them the pasteurized milk cheese did not develop as much flavor as the companion raw milk cheese developed during the course of the experiment. It was not possible to correlate the rate of development of flavor or the amount of flavor in the raw milk cheeses with the total number of non-lactic bacteria.

TABLE 1

*Characteristics of representative cultures of micrococci isolated from raw milk cheese*

Characteristic	Group no.					
	1	2	3	4	5	6
Litmus milk*	a, r, c, s	a, r, c	p	sl. a	a, r, c, s	n to a, r, c, p
Pigment**	W	W	W	W	W	Y
Tributyrin lipolysis	+	+	+	-	-	+
Butterfat lipolysis	-	+	-	-	-	- to +
Proteolysis (milk agar)	-	-	+	-	+	+
Growth at pH 5.2	+	+	- to sl.	-	+	- to sl.
Survival of pasteurization	var.	-	-	-	-	+

\* a = acid  
r = reduced  
c = coagulated  
p = peptonized  
n = no change  
s = shrunken curd

\*\* W = white  
Y = yellow or buff-colored

Over 600 cultures were isolated from the tryptone agar plates from the raw milk cheeses. Six groups of micrococci were isolated on the basis of the characteristics selected as indicative of importance in cheese ripening. These groups accounted for 78 per cent of the colonies that were picked; the remaining 22 per cent were coliforms and miscellaneous types. All of the micrococci were Gram-positive and catalase-positive and grew well in 6.5 per cent sodium chloride. They were non-pigmented except for group 6 and all of them were large cells

TABLE 2

*Distribution of groups of micrococci and other bacteria isolated from raw milk cheese*

Group no.	Total isolates	Cheeses in which group was found
	(%)	(%)
1	25	100
2	13	75
3	13	34
4	5	50
5	7	40
6	15	75
Coliforms and misc. types	22	100

occurring in tetrads or irregular masses. No growth occurred in broth containing sodium azide. The chief differences in these groups are recorded in table 1 and the distribution of these organisms in the raw milk cheeses is recorded in table 2. Several colonies were isolated from the plates made on the pasteurized milk cheeses. Most of these colonies were yellow pigmented micrococci similar to those in group 6.

Representative cultures from each group of micrococci were identified as closely as possible by the use of the differential characteristics suggested by

TABLE 3

*Effect of Streptococcus lactis on the growth of micrococci in skim milk at 30° C.*

Culture no.	Group no.	Micrococcus count when equal inocula of <i>S. lactis</i> and <i>Micrococcus</i> sp. were used	
		Initial	2 d.
(All counts $\times 1000$ )			
273	1	13,900	170,000
325	1	8,000	140,000
361	1	1,500	35,000
556	1	2,400	30,000
396	2	700	2,400
552	2	1,300	< 100
572	2	750	14,000
651	2	1,300	200
476	3	3,100	250
729	3	215	1,300
557	4	90	2
688	5	1,000	820
298	6	6,000	< 100
627	6	400	400
722	6	7,000	< 100



Hucker (5) and Bergey's Manual (1). The members of groups 1, 2 and 4 were most closely related to *Micrococcus freudenreichii*; groups 3 and 5 were most closely related to *Micrococcus caseolyticus*; and group 6 was identified as *Micrococcus conglomeratus*.

The results of attempts to grow representative organisms from each group

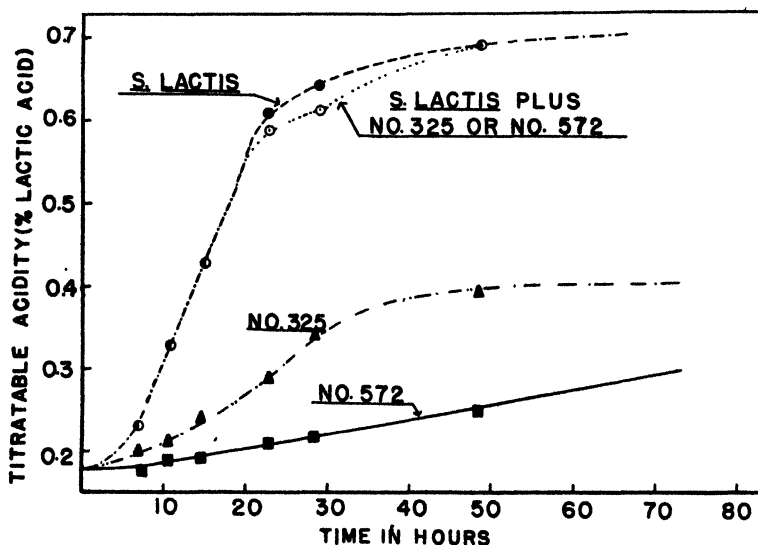


FIG. 3. Development of acidity in skim milk at 30° C. by pure and mixed cultures of micrococci and *Streptococcus lactis*.

with *Streptococcus lactis* are recorded in table 3. All of the cultures from group 1 and about half from group 2 showed a decided increase in count after 2 days of incubation with *S. lactis*. Only one culture in group 3 and none in the remainder of the groups showed an increased count after 2 days.

The effect of representative cultures on the production of acidity by *Streptococcus lactis* is given in figure 3. It is apparent from these data that the addition of micrococci would have little effect on the development of acidity during the manufacture of the cheese.

#### DISCUSSION

Comparison of the flora of raw milk cheese with that of pasteurized milk cheese indicates that the non-lactic bacteria are present in sufficient numbers in cheese made from raw milk to be of significance in its ripening. Yale and Marquardt (10) found that 10,000,000 coliforms per g. would cause sufficient development of gas to affect the quality of the cheese. If this number of coliform bacteria can bring about such a definite chemical change in the matter of a few hours, it is reasonable to assume that a smaller number of organisms could bring about the changes resulting in the development of flavor.

Although the micrococci isolated from cheese were different in many respects, they all were classified as *M. freudenreichii*, *M. caseolyticus*, or *M. conglomeratus*.

Some of the characteristics that were used to indicate the possible importance of the micrococci in cheese ripening were not necessary for the differentiation of species. This emphasizes the difficulty of classification of cultures for specific purposes or showing unusual characteristics on the basis of conventional differential tests. However, the present knowledge of bacterial taxonomy has not clearly indicated what constitutes a fundamental characteristic for differentiation of species and what should be considered as a characteristic of incidental value; therefore, any additional arbitrary designation of new species would only add to the present confusion.

The growth of micrococci of groups 1 and 2 at a pH less than 5.5 and the ability of these organisms to grow appreciably in the presence of *S. lactis*, as well as their lipolytic ability, indicates that they might be of value in increasing the rate of flavor development when added to pasteurized milk cheese. The results of investigations in which the micrococci were added to pasteurized milk for cheese making will be reported in a subsequent paper.

#### SUMMARY AND CONCLUSIONS

1. By the use of a tryptone agar medium that would not support good growth of the lactic acid bacteria, it was possible to show that cheese made from raw milk contained 500,000 to 50,000,000 non-lactic bacteria per g. by the time the cheese was 2 days old. The maximum counts obtained on cheese made from the same lot of milk that had been pasteurized were never more than 50,000 per g.

2. In raw milk cheese that developed a good flavor, micrococci were the predominant non-lactic organisms present in the early stages of ripening.

3. Seven groups of micrococci were isolated and separated on the basis of characteristics indicative of potential value in cheese ripening. These organisms were identified as *Micrococcus freudenreichii*, *Micrococcus caseolyticus*, and *Micrococcus conglomeratus*. Certain strains of *M. freudenreichii* grew in the presence of *Streptococcus lactis* and had other characteristics indicating that they might be involved in the ripening of cheese made from raw milk.

#### ACKNOWLEDGMENTS

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# EFFECT OF MICROCOCCI ON THE DEVELOPMENT OF FLAVOR WHEN ADDED TO CHEDDAR CHEESE MADE FROM PASTEURIZED MILK<sup>1</sup>

JOHN A. ALFORD<sup>2</sup> AND W. C. FRAZIER

*Department of Agricultural Bacteriology, University of Wisconsin, Madison*

There have been relatively few experiments in which selected strains of micrococci were added to cheese milk before it was set in an effort to increase the rate of ripening. Evans *et al.* (3) were able to show some improvement in flavor development in cheddar cheese when micrococci were added to the pasteurized milk in conjunction with streptococci and lactobacilli. Hansen (6) and Harris and Hammer (7) obtained an increased flavor production with selected strains of micrococci in cheddar cheese. Deane (2) isolated an acidoproteolytic micrococcus from a 4-yr. old cheddar cheese and added it, along with the regular lactic starter, to cheddar cheese made from raw milk; he found a markedly improved flavor development. Gorini (5) has emphasized the importance of acidoproteolytic cocci in cheese ripening. On the other hand, Hucker and Marquardt (8) obtained a bitter flavor when they added a proteolytic micrococcus to the cheese milk.

The results of the investigations on the growth and physiology of the different groups of micrococci isolated from raw milk cheese, reported in an earlier paper (1), indicated that two groups of cultures were of possible importance in cheese ripening. These two groups, which accounted for 38 per cent of the cultures isolated, were found in nearly all of the raw milk cheeses examined, and only occasionally were encountered in cheese made from pasteurized milk.

The present investigation was concerned with the effect of the addition of these selected micrococci on the development of flavor in cheese made from pasteurized milk.

## EXPERIMENTAL PROCEDURE

The micrococci used in this investigation were representative organisms from the two groups of micrococci that had been isolated from raw milk cheese and shown to be of possible importance in affecting the flavor of cheddar cheese (1). Both of these groups of micrococci were identified as strains of *Micrococcus freudenreichii*, although cultures in group 2 would hydrolyze butterfat, whereas those in group 1 would not.

The cultures were grown in sterilized skim milk for 24 to 30 hr. at 30° C. and added to the cheese milk with the regular lactic starter. A 1 to 3 per cent inoculum was used, the acidity of which varied from 0.25 per cent to 0.55 per cent, de-

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<sup>2</sup> Present address: Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge.

pending upon the culture being studied. The cheese was manufactured in 50-lb. vats according to recommended procedures (10). It has been shown by Knight (9) that grinding of cheese 2 to 3 wk. after manufacture hastens the development of flavor. Since the present investigation was concerned primarily with the effect of non-lactic types of bacteria on the development of flavor rather than their effect on body and texture, grinding was used to speed up the ripening process. All cheeses were stored at 5 to 7° C. during ripening.

An additional method of determining the effect of micrococci on the development of flavor was carried out as follows: Large volumes of cells were grown in an aerated carrot-liver medium (4). These cells were collected by means of a Sharples centrifuge and added to freshly ground, 2- to 3-wk. old cheddar cheese that had been made from pasteurized milk. After thorough mixing of the cheese and cells, the cheese was reground, packed in aluminum foil and waxed.

#### RESULTS

The growth in cheddar cheese of representative cultures of micrococci from groups 1 and 2, both of which are strains of *M. freudenreichii*, is recorded in figure 1. Beginning with an inoculum of approximately 1,000,000 organisms per

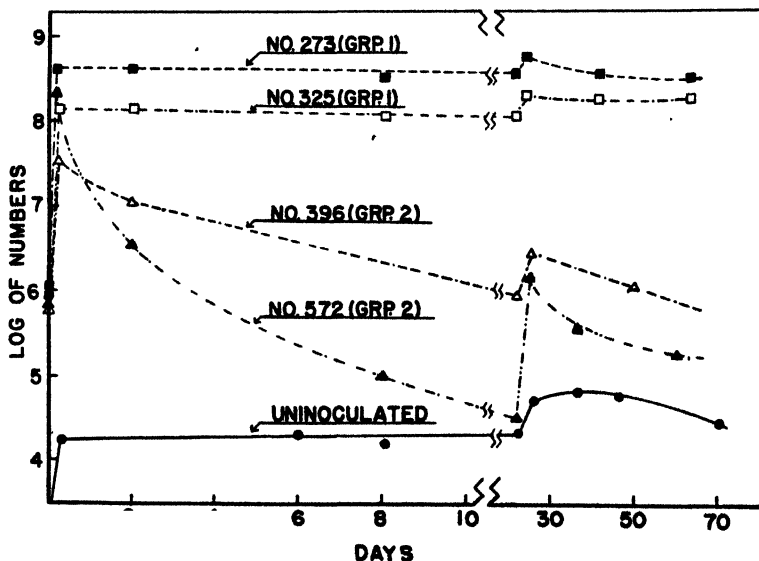


FIG. 1. Non-lactic count on pasteurized milk cheese inoculated with different strains of *M. freudenreichii*. Cheese ground at 28 d.

ml. of milk, no. 325 and 273 (group 1) increased to 200,000,000 per g. of cheese by the time the cheese was put to press. Similar increases in numbers during manufacture were shown by members of group 2 (no. 396 and 572). There was a small increase in count in the uninoculated control cheeses but it could be accounted for largely on the basis of concentration of cells in the curd.

The counts of micrococci in cheeses inoculated with members of group 1 de-

creased very slowly throughout the period of holding, although they did show a slight rise at the time the cheese was ground. When micrococci of group 2 were added to the cheese, they decreased rapidly during the first 10 days of ripening. By the time the cheese was ground there were few viable cells in the cheese. Grinding caused an increase in numbers but the micrococci again disappeared rapidly. Part of the increase at grinding may have resulted from breaking up of clumps and colonies and part probably was actual growth. It has been shown (1) that these organisms are capable of growth at the pH of cheese.

The effect of these organisms on the development of flavor in cheese is shown in figure 2. During the first few months, the development of flavor was more

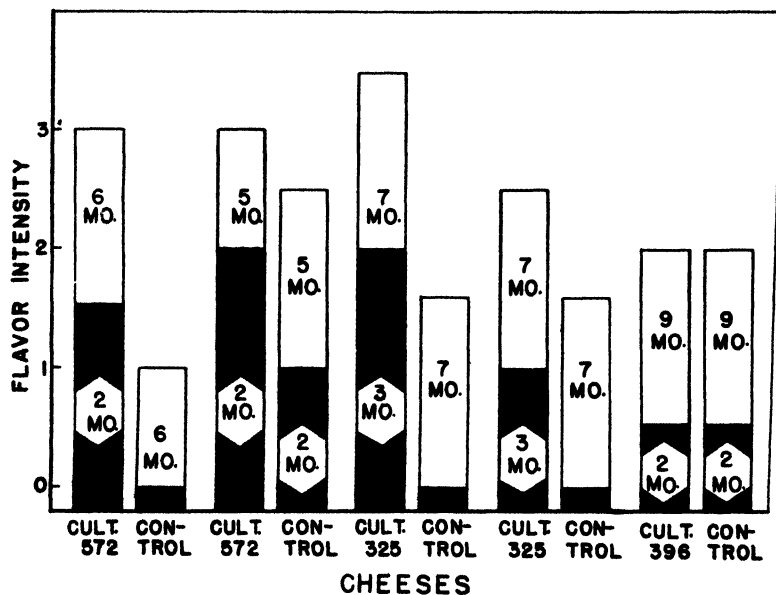


FIG. 2. Flavor development in pasteurized milk cheese inoculated at the time of manufacture with different strains of *M. freudenreichii*.

rapid in cheeses to which the micrococci were added than in the uninoculated controls. For example, in the first cheese to which culture 572 (group 2) was added, a flavor intensity of 1.5 was present in 2 mo., whereas none of the characteristic cheddar flavor was present in the control. Between 2 and 6 mo., however, both cheeses increased in flavor intensity by approximately the same amount. The other cheeses inoculated with cultures 572 (group 2) and 325 (group 1) also showed a more rapid flavor development during the early months than was found in the uninoculated control. All strains of micrococci were not effective, however, as is shown by no. 396, a member of group 2.

A few samples of the cheeses to which micrococci were added were not ground in order to check the effect of grinding on flavor development. Although the unground samples did not show as rapid or extensive development of flavor as

did the ground cheese, they did contain more flavor than similar samples of the uninoculated cheese.

It was necessary to carry the micrococcus to be used as a starter in a culture separate from the regular lactic starter. When the micrococcus was added to the regular lactic starter and transferred serially with it, the micrococcus disappeared from the mixture in four or five transfers.

The results of the addition of large masses of cells to ground cheese is shown in figure 3. The heavy inoculum of cells resulted in a rapid development of

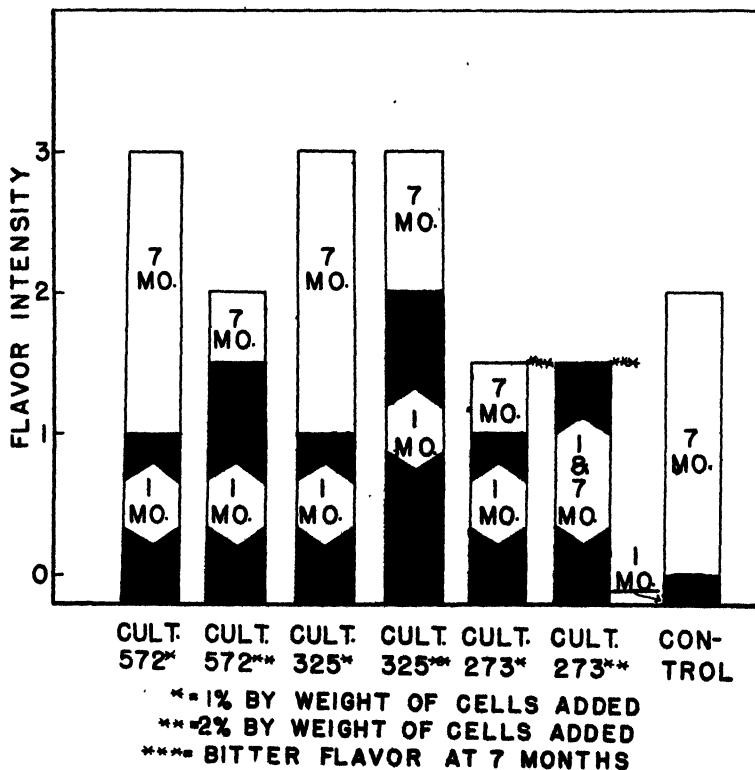


FIG. 3. Flavor development in pasteurized milk cheese ground and inoculated with strains of *M. freudenreichii* at the age of 2 wk.

flavor in all cheeses as compared to the control cheese. With culture no. 273, however, a bitter flavor developed on continued holding. This suggests that certain desirable micrococci must be added in smaller numbers than others to avoid the development of undesirable flavors. These data support those in figure 2 and indicate that the development of flavor in cheese containing certain strains of *M. freudenreichii* was faster than in cheeses to which only a lactic starter had been added and that this effect was most pronounced during the early months of ripening. The total plate count on tryptone agar in these cheeses amounted

to 1 to 5 billion cells per g. immediately after the addition of the inoculum. Culture no. 572 (group 2) disappeared rapidly as it had done when added to the cheese milk, whereas no. 325 and 273 (group 1) displayed the typical slow decline.

Total volatile acidity and water-soluble nitrogen in the cheese could not be correlated with the effect of the micrococci on flavor development.

#### DISCUSSION

The results of this investigation substantiate those of other workers by indicating that micrococci may improve the flavor of pasteurized milk cheese. Some strains of *M. freudenreichii* disappeared very rapidly from the cheese when added during manufacture yet caused an improvement in flavor. Other strains still were present in comparatively large numbers when the flavor had developed.

Some investigators, in studying the effect of microorganisms on cheese ripening, have attempted to separate the activity of bacteria from the activity of enzymes by establishing conditions that prevented bacterial growth. In view of present day knowledge of the activity of bacterial enzymes, it is unlikely that any such distinction can be made. Even though certain of the enzyme systems of the bacteria are blocked by the addition of chemicals and as a result the bacteria do not proliferate, it is known that the remaining enzymes will continue to act until exhausted or an equilibrium is reached. Apparently, therefore, bacteria of any group that occurs in sufficient numbers during manufacture and early ripening of cheese, may have a significant effect on the over-all ripening of cheese even though the viable count on these organisms decreases rapidly.

Certain strains of *M. freudenreichii* isolated from raw milk cheese are capable of hastening the development of flavor in pasteurized milk cheese. Undoubtedly, the micrococci are not the only bacteria involved in the development of flavor, but since they may be present in significant numbers in raw milk cheese, it is necessary to consider them as a factor in the development of the typical, but elusive, "cheddar flavor." The addition of selected strains of these organisms might be of value in improving the development of flavor in the commercial manufacture of cheddar cheese made from pasteurized milk.

#### SUMMARY AND CONCLUSIONS

1. Selected strains of *Micrococcus freudenreichii* were found to cause an increased rate of flavor development during the early months of ripening of cheddar cheese.
2. Some of the strains of *M. freudenreichii* that caused an accelerated rate of flavor development increased to their maximum numbers by the time the cheese was placed in the press, then decreased slowly over a period of several months. Other strains showed a similar increase during manufacture, then rapidly decreased in numbers. Both of these types of micrococci produced an increase in the rate of flavor development in cheese made from pasteurized milk.
3. The addition of large masses of micrococci to pasteurized milk cheese when it was ground at the age of 2 to 3 wk. resulted in a rapid development of flavor during curing at 5 to 7° C.



## ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of W. V. Price in the manufacture, packaging, and determination of flavor in the cheeses, and the assistance of W. C. Winder and A. R. Swanson in judging the cheeses.

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# INHERITANCE OF SUSCEPTIBILITY TO MASTITIS<sup>1</sup>

JAY L. LUSH

*Animal Breeding Subsection, Iowa Agricultural Experiment Station, Ames*

Clinical reports and family histories which indicate some hereditary basis for susceptibility to mastitis are numerous. Most of these concern isolated cases which came to attention merely because they seemed so unusual. Murphy *et al.* (1) have reviewed this literature. Ward (2) has published preliminary results from nine herds surveyed systematically so as to gather all the information, whether conspicuous or not.

The present note is to call attention to some data which show that inheritance plays a part worth attention and to a method of measuring roughly how much the hereditary differences in susceptibility to mastitis have to do with determining whether or not an individual cow develops mastitis.

## SOURCE OF DATA

The data were printed on page 60 of the 21st Annual Report of the New Zealand Dairy Board, which is for the year ending July 31, 1945 (3). The data came from 15 herds in the Canterbury District and 12 herds in the Manawatu District, chosen because they were conveniently located and the owners were especially interested in mastitis. Cooperating farmers were asked to keep full details of all clinical cases of mastitis occurring and to notify the Consulting Officer immediately. The working definition of clinical mastitis was: "All quarters which are abnormal or which are giving abnormal milk. This includes any quarters showing discoloured milk, clots, sediment, or watery milk; also any quarters showing hardness, pain, swelling, or other similar abnormal condition." The details concerning the leucocyte counts, fraction of cases in which streptococcus or staphylococcus organisms were found, apparent connection with seasonal or managemental conditions, etc., can be read in the report. On the basis of these tests each cow was classified as "susceptible" if she developed mastitis at any age or as "resistant" if she had not developed mastitis and had reached at least 8 yr. of age. Since mastitis manifests itself in many degrees of severity, this grouping into only two classes is a bit arbitrary and doubtless loses some of the information really in the data. In this respect, however, it seems no more and no less valid than any other grouping of a continuously distributed variable into only two classes, such as high and low producers, or kept and culled.

The tables include all dams who reached at least 8 yr. and were classified as susceptible or resistant themselves and who had any daughters grow up to be classified as susceptible or resistant. The susceptible dams are listed in one column and the resistant dams in another. Opposite each group is shown the percentage of susceptibles among their daughters. This was done separately for each herd.

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<sup>1</sup> Journal Paper no. J-1718 of the Iowa Agricultural Experiment Station, Ames, Project no. 1053.

Table 1 shows, as an example, how this was done for the first six herds in the Manawatu district. Two columns are added to show how the data from all herds were combined to yield the totals at the bottom of the table.

TABLE 1  
*Sample data showing how the evidence was combined*

Herd No.	Susceptible dams		Resistant dams		$x \cdot y$	$\frac{nk}{n+k}$
	No. (n)	% daughters susceptible (x)	No. (k)	% daughters susceptible (y)		
1	23	87.0	18	66.7	20.3	10.10
2	5	80.0	6	33.3	46.7	2.73
3	17	94.2	15	66.7	27.5	7.97
4	1	100.0	2	100.0	0.0	.67
5	15	80.0	21	61.9	18.1	8.75
6	7	71.5	8	50.0	21.5	3.73
<hr/>						
Totals: <sup>a</sup>						
Manawatu	128		171		1034.48	64.06
Canterbury	86		109		919.72	35.57
<hr/>						
Averages (weighted):						
Manawatu		81.3		54.4	16.1	
Canterbury		89.5		56.0	25.9	
Pooled					19.6	

<sup>a</sup> The totals in the  $x \cdot y$  column are of the  $x \cdot y$  for each herd times its  $\frac{nk}{n+k}$ , as the best measure of the amount of information that herd contributes.

The evidence on heritability consists simply in whether susceptible dams have a higher percentage of susceptible daughters than the resistant dams in the same herd. This amounts to an intra-herd regression of daughters on dams. That is, the two groups of dams are 100 per cent apart in their phenotypic susceptibility when classified in this way. If 80 per cent of the daughters of the susceptible dams were susceptible while only 60 per cent of the daughters of the resistant dams were susceptible, the average susceptibility of the two groups of daughters would differ by 20 per cent, whereas their dams differed by 100 per cent. Such an outcome—unselected daughters differing only one-fifth as much as their individually selected dams—would indicate that the heritability of this phenotypic difference between individual dams was two-fifths, since it is assumed that the sires of the two groups of daughters would be about equal to each other in anything they would transmit in regard to susceptibility to mastitis and, therefore, that the phenotypic averages of the unselected daughters would differ by only half as much as the genic averages of their dams. Indeed putting the analysis on an intra-herd basis means that in many cases the very same individual bulls were sires of daughters from the resistant and also from the susceptible dams.

The mean incidence of mastitis appears to vary widely from herd to herd but those differences do not affect the interpretation here<sup>2</sup> since the  $x \cdot y$  column con-

<sup>2</sup> Except in choosing a suitable weighting scale for combining the evidence from the different herds.

cerns only intra-herd differences. If the intra-herd environmental differences which influence whether a cow comes down with mastitis were randomized as between daughter and dam, the regression of daughter on dam should show only a genetic relationship plus some sampling variations from Mendelian segregation and from the impact of those intra-herd environmental differences not being wholly equalized in numbers of cows as few as these. Doubling  $x$ - $y$  yields an estimate of how much of the phenotypic difference between the dams being "susceptible" and "resistant" was due to genic (= additively genetic) differences between them, plus possibly a bit of the epistatic effect if any. This estimate is subject to considerable sampling error because the data were moderately few.

Some method of weighting is necessary for combining the evidence from the various herds, since the herds varied widely in the total number of dams which were classified and also in the proportion of dams in the two groups. The weight

$\frac{nk}{n+k}$  was chosen as proportional to the amount of information from each herd, since it is proportional to the inverse of the variance if the true proportions of susceptibles among the daughters really were the same in all the herds. Weight-

ing in proportion to  $\frac{nk}{ny(100-y) + kx(100-x)}$  would have seemed more appropriate if one could have assumed that the observed  $x$  and the observed  $y$  were really typical for each of the herds involved, but that method was rejected as unsuitable because of the small numbers and especially in view of the difficulties which arise with it when either  $x$  or  $y$  is 100 or zero. An angular transformation seemed unproductive for percentages with such small denominators as these. The method used may have underemphasized a bit the evidence from herds in which either  $x$  or  $y$  was zero or 100 per cent but it is not believed that the bias is serious, especially when compared with the sampling errors.

The average intra-herd regression of daughter on dam, using this method, was 0.161 for the 12 Manawatu herds and 0.259 for the 15 Canterbury herds. The average for all 27 herds was 0.191. The standard deviation of this estimate would be not far from 0.08, since the sum of the weights is equivalent to about 100 susceptible dams and 100 resistant dams, if  $n$  and  $k$  were alike in the same herd although variable from one to another of the 27 herds. Therefore the  $P = 0.05$  fiducial limits for the regression of daughter on dam would be about 0.16 above or below the observed 0.19. This yields an estimate that heritability of individual differences in susceptibility to mastitis is about 0.38 but that the 95 per cent confidence interval for this would range from in the neighborhood of 0.06 up toward 0.70. In short, the evidence is good that heredity plays some part and the unbiased indication is that it is a moderately large part in whether a cow develops mastitis, but the data are too few to locate that within narrow limits.

#### DISCUSSION AND QUALIFICATIONS

The all-or-none nature of the classification tends to make the regression less than would be found if susceptibility to mastitis could be measured in many dif-

ferent grades. This effect is small where the two groups of dams are as nearly equal in numbers as they were here.

That the standards for classifying the animals into susceptible and resistant groups were rather severe is indicated by the fact that 42 per cent of the dams and about 70 per cent of the daughters were classified as susceptible. Since the dams were all at least eight years old, prior culling of some cows on mastitis explains partially the lower figure for the dams. The rest of the explanation is that daughters which were under 8 yr. of age and had not developed mastitis were merely omitted from the data on the grounds that they might yet develop it before reaching eight. Had these daughters been included as resistant (personal communication from Ward and Castle in 1949) the percentages of susceptible daughters would have been 66 and 44 instead of 81 and 54 in Manawatu and would have been 67 and 43 instead of 89 and 56 in Canterbury. This would not have changed the general conclusion.

How far one can generalize from these data is uncertain, especially in view of the wide fiducial limits. They do agree well with the reports reviewed by Murphy *et al.* (1). The herds in this New Zealand study included some with high and some with low incidence of mastitis but presumably their average incidence was a bit higher than in the general population in New Zealand, as the owners were interested in the problem enough to cooperate thoroughly with the Consulting Officers. The difference between the Canterbury average and the Manawatu average is only about the size of its standard error, so there is little indication here that the two sets of herds really differed. Some causes of mastitis which might be important in other countries are presumably absent from or negligible in New Zealand dairying where the herds are on pasture all the time and there is little or no opportunity for injury in barns or for damage from lying on cold floors.

This method of investigation appears worth extending to a much larger body of data. The wide fiducial limits found for the heritability value indicate how many herds need to be included for those limits to be narrowed as much as it is desirable that they should be. To get the standard error of the regression as low as 0.04 would require about 400 susceptible and 400 resistant dams.

Whether a heritability value of about 0.4 appears unreasonably high depends obviously, on one's prior opinions. Insofar as ability of the cow to combat and overcome the disease depends on the anatomical structure of the mammary gland, a value this large appears reasonable, since many details of anatomical structure are highly hereditary. So far as the cow's overcoming the disease depends on her general state of health, a value of 0.4 appears surprisingly high, since general health is affected by so many external circumstances.

If heritability really is this high, culling of the affected individual cows and paying a moderate amount of attention to not using bulls whose sisters or dams were susceptible should reduce the incidence of mastitis in dairy herds rather rapidly per cow generation. Since the parents average about five years old when the dairy calf is born, even a rather rapid amount of such genetic improvement per generation might appear disappointingly slow to one who has mastitis widely

prevalent in his herd at a given time. The fact that mastitis is still so prevalent and that mass selection should be reasonably effective if heritability is this high and that selection against low production must automatically involve considerable selection against mastitis, makes it seem doubtful that heritability is in fact as high as .4. Or perhaps the same genes or anatomical structures which predispose to susceptibility to mastitis may also predispose to high production or to some other characteristic for which selection is positive. This question of intercorrelations (either genetic or environmental or both) between characteristics is so speculative at the moment that further discussion of it here seems fruitless. Yet it remains clear that daughter and dam do resemble each other in susceptibility to mastitis closely enough that culling those most affected should lessen the incidence and severity of mastitis.

Ward and Castle have used the present method in a preliminary study of fertility in 12 herds (personal communication in 1949) and have found intra-herd daughter-dam regressions of 0.08, 0.08, and 0.15 according to whether the daughters were classified as infertile when they were (a) empty their first year, or (b) empty either of their first two years, or (c) empty either of their first three years. The dams were classified according to their performance in a five-year period before they were nine years old. If the dams calved in all five years or if their fertility index was less than two services per conception they were classified as highly fertile, while if they were empty one or more of the years or if their fertility index was higher than two services per conception they were classed as of low fertility. Since the numerator and the denominator of the regression coefficient in this case are not in exactly the same units, some adjustment for the variation not being alike in the two kinds of units might need to be made if these regressions are to be converted into estimates of heritability. Such adjustment would be small since both units are percentages in a two-way classification.

#### SUMMARY

The average intra-herd regression of daughter on dam within 27 herds in New Zealand was 0.19 for whether they came down with mastitis. Since the 95 per cent confidence interval for this is of the order of 0.03 to 0.35 its true magnitude in the population from which these data are a sample is not known closely; yet it appears that differences in susceptibility to mastitis have a strong genetic background. Selection against cows which are severely affected or have severely affected sisters or daughters should lower the incidence of mastitis. The method of investigation appears worth extending to more data.

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# THYROPROTEIN IN THE RATION OF DAIRY CATTLE. I. ITS INFLUENCE ON MILK PRODUCTION, FAT TEST, HEART RATE AND BODY WEIGHT<sup>1</sup>

RALPH P. REECE

*Department of Dairy Industry  
New Jersey Agricultural Experiment Station, New Brunswick*

## INTRODUCTION

In considering the possibilities of feeding thyroprotein on a commercial basis, it is important to know whether sufficient thyroxine is secreted in the milk to affect the consumer. In producing milks for such a study, certain observations were made on cows fed thyroprotein and others not receiving thyroprotein. These observations, along with results on additional animals, are presented in this paper. The results on the milk study have been reported elsewhere by Bruger *et al.* (1).

## EXPERIMENTAL PROCEDURE

Four dairy cows past their lactational peaks each received 15 g. of thyroprotein (Protamone<sup>2</sup>) daily for 16 wk. No attempt was made to select cows that might respond well to thyroprotein feeding. The thyroprotein was incorporated in the morning grain ration. During the entire feeding period, daily milk weights (twice-daily milkings) were recorded and milk samples were taken on 2 consecutive days every other week. Individual milk samples were tested for their butterfat content (Gerber method) and solids-not-fat content (by means of a lactometer). In an attempt to determine the influence of thyroprotein feeding on milk production in each cow, the rate of decline prior to thyroprotein feeding was determined and the lactation curve projected on this basis. Body weights and heart rates (measured with a stethoscope) were determined on 2 consecutive days every other week. Similar observations were made on four additional cows that did not receive thyroprotein. This group of cows cannot be considered strictly as a control group because the groups were not well balanced. The observations are presented, however, for comparison. These observations all were made during the barn-feeding period. Fifteen g. of thyroprotein were fed daily for various periods, to an additional four cows on which observations were made only on milk production. The cows were maintained under average farm conditions, grain being fed according to milk production. A description of the cows on which observations were made is given in table 1.

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<sup>2</sup> The Protamone was generously supplied by the Cerophyl Laboratories, Kansas City, Missouri, through the courtesy of W. R. Graham, Jr.

TABLE 1  
Description of cows on which observations were made

Cow no.	Breed	Age	Received thyro- protein	Mo. of lactation	Mo. of ges- tation
		Yr. Mo.			
150X	Holstein-Fr.	12-2	Yes	5th	0
462	Ayrshire	9-9	"	4th	0
491	Ayrshire	5-9	"	6th	4th
637	Brown Swiss	2-10	"	5th	3rd
H-60	Holstein-Fr.	7-10	No	7th	2nd
626	Brown Swiss	5-5	"	8th	5th
628	Brown Swiss	5-1	"	2nd	0
467	Ayrshire	8-10	"	2nd	0
H-56	Holstein-Fr.	4-1	Yes	8th	5th
647	Brown Swiss	9-5	"	13th	0
648	Brown Swiss	5-7	"	6th	1
650	Brown Swiss	7-4	"	5th	0

## EXPERIMENTAL RESULTS

*Milk Production.* In thyroprotein studies the initial response is of considerable interest. In the eight cows that received 15 g. of thyroprotein for various periods, the average increase in milk production from the week prior to thyroprotein feeding to the highest subsequent weekly production was 24.8 per cent (range 7.9 to 36.2). On the average, the cows attained their highest weekly average production during the second week of thyroprotein feeding. In the tenth week daily milk production was nearly identical to that of the week prior to thyroprotein feeding (31.6 lb. and 31.7 lb.). Of the eight cows, two attained the highest production in the second week of thyroprotein feeding, two in the third week, three in the sixth week, and one in the seventh week.

Four cows were fed 15 g. of thyroprotein daily for 16 or more wk., and during the same period observations were made on four additional cows that did not receive thyroprotein. The lactation curve for the cows not fed thyroprotein indicated no unusual herd conditions. Of the four cows fed thyroprotein, three had lactation curves that eventually dropped below their estimated curves. The fourth cow (637) gave an excellent initial response and her lactation curve remained above the estimated lactation during the entire feeding period. The feeding of thyroprotein may have increased her persistency. Prior to thyroprotein feeding her average rate of decline was 7.4 per cent, whereas during the feeding it was 4.1 per cent (fig. 1-A, B, C, D & E). The thyroprotein-fed cows decreased 32 per cent in milk production from the last 2-wk. period of feeding to the first 2-wk. period after thyroprotein withdrawal. During the same period, the cows not fed thyroprotein showed a 14 per cent decrease in milk production.

The influence of feeding 15 g. of thyroprotein daily on milk production was noted in four additional cows. One of these cows was a 4-yr. old Holstein-Friesian in her eighth month of lactation. Thyroprotein was fed for a 12-wk. period and in the last 2-wk. period her average daily milk production was 2.8 lb. above her estimated production (fig. 2). The three remaining cows were Brown Swiss, of which two were not pregnant and the third was in the first month of gestation. One cow (647), showed a maximum increase in daily milk produc-



tion of 3.5 lb., based on monthly averages. Five months after the incorporation of thyroprotein in the ration the level of milk production decreased to that of

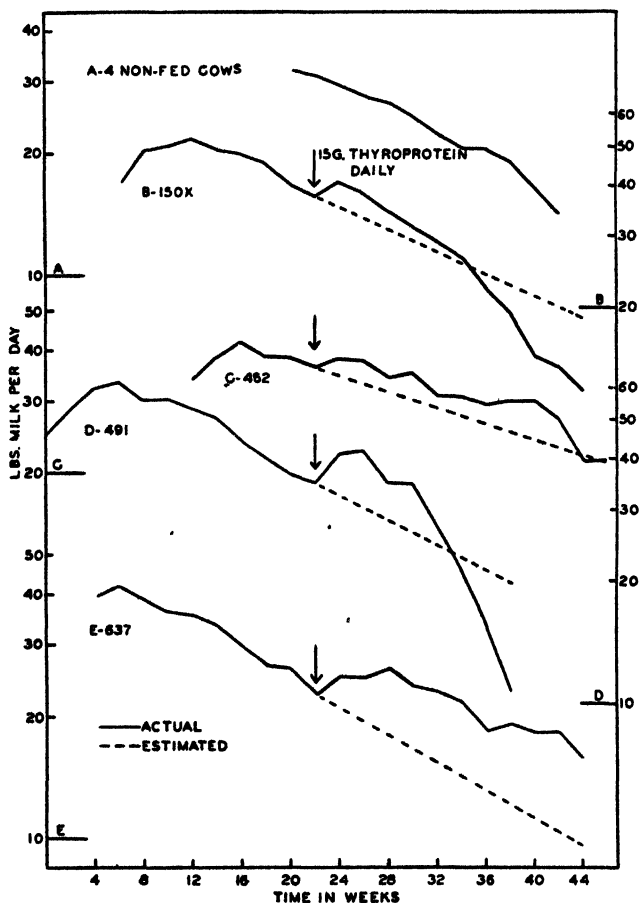


FIG. 1. The effect of feeding 15 g. of thyroprotein daily on milk production.

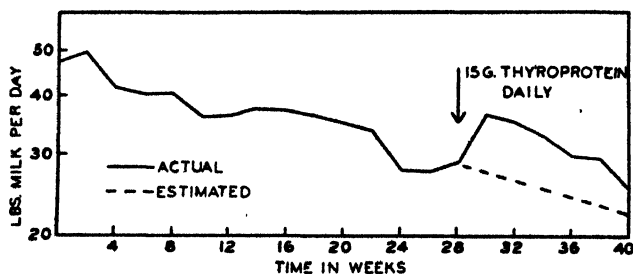


FIG. 2. The effect of feeding 15 g. of thyroprotein daily on milk production in a 4-yr.-old Holstein-Friesian cow (H-56).

her estimated production. In the seventh month of thyroprotein feeding the average daily milk production was 3 lb. higher than in the previous month. This increase in milk production may have been the result of an improvement in the nutritional condition of the animal, since the seventh month of thyroprotein feeding was her first full month on pasture following the incorporation of thyroprotein in the ration. In the ninth month of thyroprotein feeding, the 21st full month of lactation, the average daily milk production was 2.3 lb. above the estimated production (fig. 3). It was not possible to project the lactation curve of the second Brown Swiss cow (648) since there had been no decline in lacta-

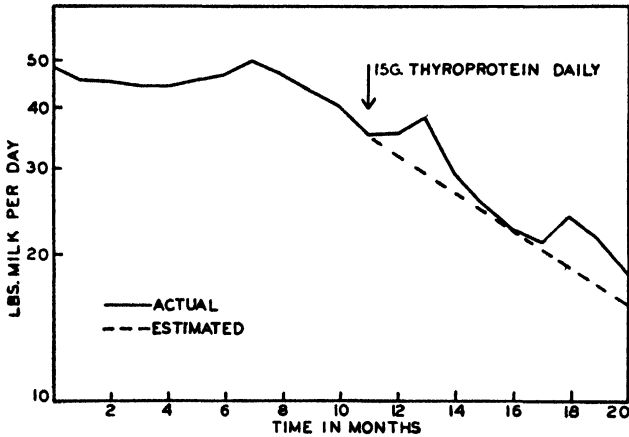


FIG. 3. The effect of feeding 15 g. of thyroprotein daily on milk production in a 9-yr.-old Brown Swiss cow (647).

tion prior to feeding thyroprotein. Based on monthly averages, daily milk production increased from 33.3 lb. to 40.8 lb. in the third month of thyroprotein feeding. The third month of thyroprotein feeding was the first full month that 648 was on pasture following the addition of thyroprotein to the ration. In the seventh month of thyroprotein feeding, and the 13th month of lactation, 648 averaged 22.1 lb. of milk daily (fig. 4). Thyroprotein was incorporated in the

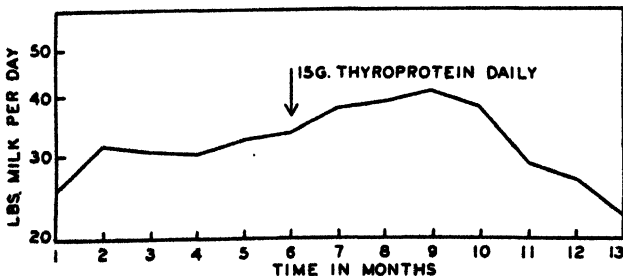


FIG. 4. The effect of feeding 15 g. of thyroprotein daily on milk production in a 5-yr.-old Brown Swiss cow (648).

ration of a third Brown Swiss cow (650) in the sixth month of lactation. Average daily milk production increased from 34.4 lb. to a maximum of 43.5 lb. in the second month of thyroprotein feeding. During the subsequent 6 mo. there was a decline in milk production, the decline being similar to that of the estimated lactation. In the eighth month of thyroprotein feeding, and the thirteenth month of lactation, the average daily milk production was 25.2 lb., or 8.9 lb. above the estimated production (fig. 5).

**Fat test.** The butterfat test of the thyroprotein-fed cows increased from 3.69 to 4.37 per cent and then decreased to 4.09 per cent, at which level it was

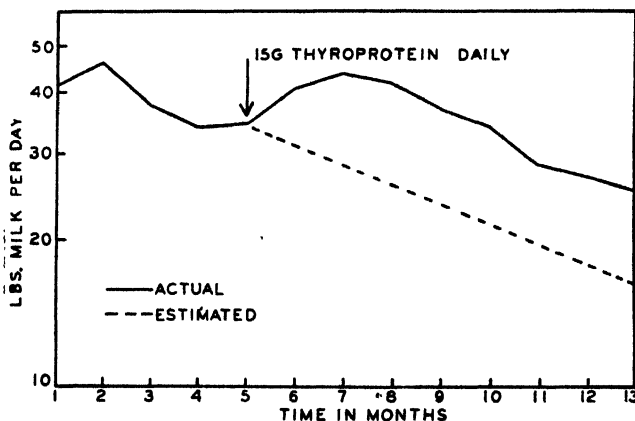


FIG. 5. The effect of feeding 15 g. of thyroprotein daily on milk production in a 7-yr.-old Brown Swiss cow (650).

maintained for the remainder of the feeding period. The butterfat test of the cows not fed thyroprotein remained fairly constant for 8 wk., after which it increased (fig. 6-A).

**Solids-not-fat.** The solids-not-fat content of the milk of thyroprotein-fed cows increased and then decreased slightly. At the end of the 16-wk. feeding period the solids-not-fat content was similar to that at the beginning of the trial. In the cows not fed thyroprotein the solids-not-fat content fluctuated somewhat; however, there was a slight increase (fig. 6-B).

**Heart rate.** In the cows fed thyroprotein the heart rate increased from 62 to 86 beats per min. and remained fairly constant for 12 wk. Heart rate then decreased to 78 beats per min. and in the final 2-wk. period the average heart rate was 82. In the cows not receiving thyroprotein, heart rate decreased slightly and then increased to within three beats per min. of the initial level (fig. 6-C). In the 2-wk. period following the withdrawal of thyroprotein from the ration the cows showed a decrease in heart rate of 17 beats per min. The cows that had not been fed thyroprotein showed an increase of 3 beats per min.

**Body weight.** In the cows receiving thyroprotein the average body weight decreased from 1,198 lb. to 1,126 lb. and then steadily increased to 1,175 lb.

During the same period, the body weight of the cows not receiving thyroprotein increased from 1,173 lb. to 1,243 lb. In the last half of the feeding trial the weight increases in the two groups paralleled each other (fig. 6-D). In the 2-wk. period following the withdrawal of thyroprotein from the ration, the cows gained, on the average, 53 lb., whereas the cows that had not received thyroprotein showed no increase in weight.

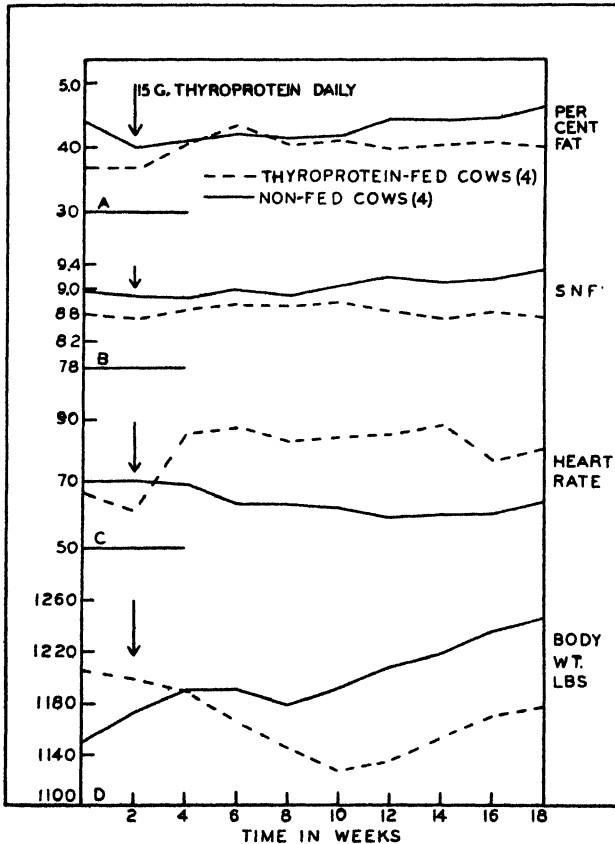


FIG. 6. The effect of feeding 15 g. of thyroprotein daily for 16 wk. on fat test, solids-not-fat, heart rate and body weight.

**Reproduction.** Of the eight cows that were fed thyroprotein four were pregnant at the beginning of the feeding period. These four cows had gestations, parturitions and calves that were normal. One cow (650) had been bred three times prior to thyroprotein feeding and had not conceived. She conceived on the fifth service during thyroprotein feeding and gave birth to a normal calf at the end of a normal gestation period. Another cow (647) had failed to conceive following ten services before being fed thyroprotein. She conceived on the third service after the incorporation of thyroprotein in the grain ration. Her

gestation, parturition and calf were normal. Two cows were open during the entire thyroprotein feeding period. One of these cows (462) was bred twice during the feeding period, but she did not conceive. She conceived on the first service after thyroprotein withdrawal. At the end of a 265-day gestation period no. 462 gave birth to a calf that appeared normal except for size (birth weight 20 lb.). The cow was negative to the blood test for Bang's disease.

#### DISCUSSION

Of the eight dairy cows fed 15 g. of thyroprotein, all showed an initial response to thyroprotein feeding. The response, however, was variable. This is in agreement with other investigations. Not all of the cows continued to produce above their estimated production. Lactation curves were projected for seven cows. Four of the curves remained above and three dropped below the estimated production. Of the four cows that maintained lactation curves above the estimated curves, three were Brown Swiss and the fourth was a Holstein-Friesian that received thyroprotein for a 12-wk. period. Of the three cows whose lactation curves dropped below the estimated curves, one was a Holstein-Friesian (150X) in rather poor physical condition at the start of the feeding trial, and two were Ayrshires. If actual and estimated lactation curves are plotted on semi-log paper, it is believed that one can determine fairly well when it is advantageous to continue the feeding of thyroprotein and when it is advantageous to withdraw it from the ration.

The fat content of the milk of the thyroprotein-fed cows increased from 3.69 per cent to 4.37 per cent. This increase in the fat content of the milk accompanied a decrease in body weight. Although body weight continued to decrease, the fat content of the milk was not maintained at the 4.37 per cent level. During the last 8 wk. of the feeding period there was an increase in body weight; however, the fat content of the milk remained fairly constant. The difference in the fat content of the milk, prior to, and at the end of the thyroprotein feeding period was about the same in the thyroprotein-fed cows and in the cows not receiving thyroprotein.

There was some indication that the solids-not-fat content of the milk may have been increased initially by thyroprotein feeding. After 8 wk. of thyroprotein feeding the solids-not-fat content of the milk returned to the pre-feeding level. On the other hand, the solids-not-fat content of the milk of cows not receiving thyroprotein was greater at the end of the feeding period than at the beginning. In one instance (150X) the solids-not-fat content increased from 7.84 per cent to 8.12 and then decreased to 6.87 per cent, from which figure it increased to 7.37 per cent.

#### SUMMARY

The daily feeding of 15 g. of thyroprotein to eight cows resulted in an initial increase in milk production of 24.8 per cent (7.9 to 36.2). Of seven cows in which lactation curves were projected, four remained above and three dropped below their estimated production.

Thyroprotein feeding increased the fat content of the milk from 3.69 to 4.37

per cent. The fat test then decreased to 4.09 per cent at which level it was maintained until the end of the feeding period.

The solids-not-fat content of the milk increased and then decreased slightly during a 16-wk. feeding period.

The average body weight of four cows decreased from 1,198 lb. to 1,126 lb. and then steadily increased to 1,175 lb.

Heart rate increased from 62 to 86 beats per min. and remained fairly constant for 12 wk.

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## COMPOSITIONAL QUALITY OF MILK. I. THE RELATIONSHIP OF THE SOLIDS-NOT-FAT AND FAT PERCENTAGES

G. A. RICHARDSON<sup>1</sup> AND A. H. FOLGER<sup>2</sup>

*With the technical assistance of C. L. Hankinson<sup>3</sup>  
University of California, Davis*

Economists, nutritionists, regulatory officials, processors of dairy products and others need to know the percentage concentration of the main constituents in milk in order to evaluate it properly for any specific use. The dairy breeder and the producer, in the past, have measured the worth of the dairy cow on the basis of the production and the fat content of her milk. But unless the true value of milk, as determined by its composition and cost of production, is made the basis of pricing plans, it would seem impossible to set up an entirely equitable method of paying for it.

Price structures, based on the fat and the skim solids percentages, the latter calculated from the fat percentage by means of equations, are criticized as being unfair to the milk of one breed or another. There is justification for this criticism. Equations showing the relationship between fat and solids-not-fat are seldom in agreement and the view is developing that no one equation is adequate for milks of widely different fat contents.

The wide variability in the percentage composition of cow's milk is recognized universally. That the specific and perhaps the significant value of milk may reside in the non-fat solids is a more recent recognition. Most legal definitions of milk include a statement of minimum fat, total solids and/or solids-not-fat. A wide disparity exists in these standards; some of them are quite illogical.

Milk fat, being easily and reasonably accurately and rapidly determined, has been the most important single unit on which to place an economic value on milk. The standard, or standards, by which to evaluate milk in the modern concept, however, must give consideration to the fact that the ratio of fat percentage to solids-not-fat percentage is not constant for milks of different fat contents.

Tocher (26) using data secured from the analyses of the milks from single milkings of 676 cows, the majority of which were Ayrshires, established regression equations showing the relationship between the various components. This survey revealed "a uniform rise in the average percentage of solids-not-fat with

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<sup>1</sup> Present address—Dept. of Dairy Husbandry, Oregon State College, Corvallis.

<sup>2</sup> Present address—Healdsburg, Cal.

<sup>3</sup> Present address—North American Creameries, Inc., Paynesville, Minn.



ascending values of percentage of butterfat"; the butterfat and solids-not-fat could be represented, however, not by normal curves, but rather by Pearson's type IV curves.

The present status of the problem is illustrated in fig. 1. Curves A-A and

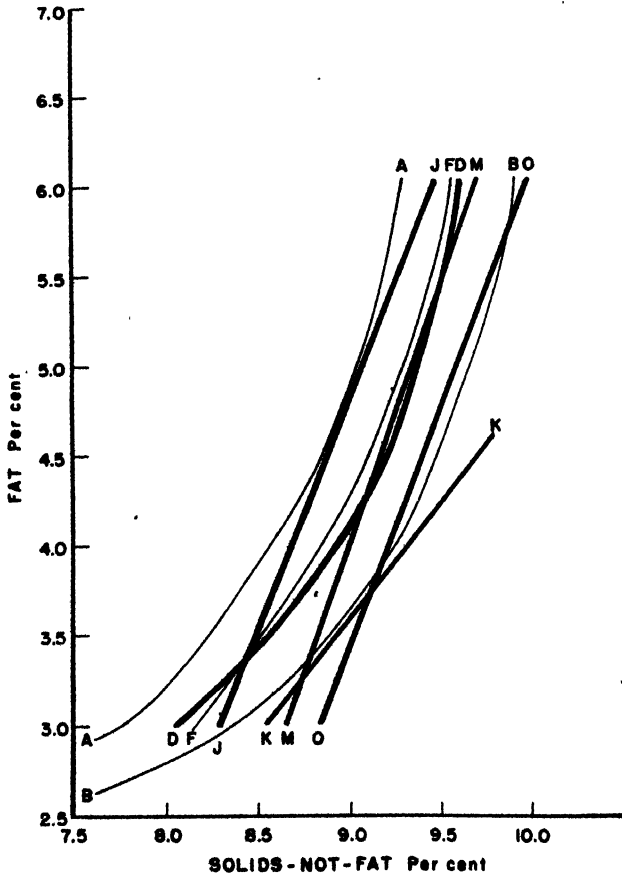


FIG. 1. The relationship between fat and solids-not-fat as shown in some representative previous studies.

*Legend for Fig. 1*

- AA-BB —zone representing 200,000 samples of shipper's milks (5)
- D-D —from representative means (5)
- F-F —from a recent compilation (6, 17)
- J-J —per cent solids-not-fat =  $0.4\% \text{ fat} + 7.07$ . (12)
- M-M " " " " " =  $0.346\% \text{ fat} + 1.597$ . (16)
- O-O " " " " " =  $0.3846\% \text{ fat} + 7.6736$ . (21)
- K-K " " " " " =  $0.7841 (7.8908 + \% \text{ fat})$ . (18)

B-B are boundaries of a zone within which fell the fat and solids-not-fat analyses of 200,000 samples of herd milks, believed genuine and unadulterated, as reported

by Brown and Ekroth (5). Curve D-D represents their analyses of 1,000 samples of genuine milks from individual cows. This latter is paralleled closely by curve F-F which represents the solids-not-fat and fat relationships tabulated by the Milk Industry Foundation (17) which, in turn, is identical with those proposed to the American Dairy Science Association by the Committee on Standardization of Market Milk (6).

In contrast to these non-linear relationships, the curves J-J, M-M, O-O and K-K, drawn from the respective regression equations, show uniform increases of approximately 0.04, 0.035, 0.038 and 0.078 per cent in solids-not-fat for each increase of 0.1 per cent in fat. As will be shown in fig. 2, Overman *et al.* (21) derived other equations to represent the milks of the major breeds in which the general linearity is not evident. The values for solids-not-fat reported by Jacobson (12) for fat values between 4.0 and 6.0 per cent coincide closely with those reported by Ilaecker (9). The latter reported lower values corresponding to fat values below 4.0 per cent than those established by Jacobson.

In compositional studies of the nature indicated, as well as in the several others to which reference has not been made, comparatively little attention seems to have been given to the role of abnormal udder tissue and heredity. In a recent report, Rowland (24) placed emphasis on feed and breed family strain and minimized the role of mastitis. Kay (14), however, earlier attributed about 80 per cent of their poor quality milks to mastitis. This latter conforms closely to the authors' data (unpublished). Moore and co-workers (18, 19) obtained evidence that fat and solids-not-fat are inherited separately and that the daughters of a given sire may produce milk higher, lower or unchanged from that of their dams. Nicholson and Lesser (20), however, from the results of a 3-yr. study of nearly 5,000 samples from one herd and over 600 from another herd, the former being given partial statistical analysis, reported for one herd that it was not possible to trace the low level of solids-not-fat to any particular bull. They failed to account for the fact that the incidence of low solids-not-fat was higher for one herd than for another of the same breed. Although the milks normal in solids-not-fat were not restricted to cows not reacting to tests for mastitis, the incidence of mastitis among the low solids-not-fat animals was high. These authors stated, "Damage to the mammary glands resultant from previous disease or to an existing condition of mild or chronic mastitis might be considered as a possible explanation of the abnormal composition of some of these milks". Low solids-not-fat were associated with high chlorides. Milks of abnormal composition have been reported for cows with no history of mastitis (10, 11).

Although this discussion of the literature is by no means inclusive, mention should be made of a recent report from Sweden. Bonnier *et al.* (4) subjected to statistical treatment the analyses of 2,152 samples of milk, collected under supervision. They found no linear or quadratic relationship between the percentage of protein or lactose and that of fat except within intervals of 0.6 per cent fat. This suggests that linear-regression equations relating solids-not-fat to fat percentages should be used with caution. This especially is true for certain herd milks (1).

The current interest in milk marketing plans, some of which give consideration to solids other than fat and which assume that the percentage of non-fat-solids rises uniformly with an increase in the percentage of fat, prompts the publishing of data secured in 1935. Some of these cows were screened for mastitis and all were subjected to the climatic conditions of California.

#### EXPERIMENTAL

*Herds.* Herds were selected on the basis of size, accessibility and availability of accurate feeding, breeding and production records. Herd A provided from 10 to 16 purebred Holsteins and from 14 to 18 purebred Jerseys per month throughout the year. Three-day composites (proportional aliquots) of the milks of individual cows were collected each month. Herd B provided approximately 80 purebred Holsteins. Personally supervised single milkings of each individual cow were taken at irregular intervals. Herd C consisted of approximately 80 purebred Holsteins. Carefully supervised single milkings of individual cows were collected twice during the year. As time permitted, additional samples of single milkings of individual cows were collected from selected herds of purebred and grade Ayrshires and of purebred Holsteins.

*Incidence of udder abnormalities.* Initially, it was planned to secure sufficient data on sire-daughter relationships to permit statistical treatment. Early results brought out irregularities and abnormalities and suggested the possibility of mastitis as a causal factor. Tests revealed this to be the case and prompted curtailment of the number of herds tested as well as the frequency of testing. Tests for latent mastitis or for abnormalities usually associated with mastitis were considered positive if the foremilk reacted abnormally to two or more of the following tests in one or more quarters bromthymol blue, chloride, leucocyte count and the presence of long chain streptococci (22). The merely suspicious reactions were included with the negative groups. It is to be expected, therefore, that the reported incidence of abnormal udder tissue is conservative.

*Chemical tests.* The milks were analysed for fat by the Babcock method, for total solids by the Mojonnier procedure; solids-not-fat were obtained by difference. The analyses for protein, lactose and ash will be reported in a later paper.

#### RESULTS

Analyses for fat and solids-not-fat were made on 717 samples of Holstein, 231 of Jersey and 100 of Ayrshire milk. Table 1 shows the data grouped according to fairly narrow extremes of fat percentages and screened for abnormalities by using the usual biochemical and bacteriological tests for mastitis. In fig. 2, curve C-C<sub>4</sub> was constructed from the normal milks and curve P-P from the abnormal milks. If the data for cows, 20 to 25 per cent of which are abnormal (mastitic), are similarly plotted, the curve will closely parallel the C-C<sub>4</sub> curve but will be on the left of the latter. For purposes of comparison, the curves erected by Jacobson (J-J), Brown and Ekroth (D-D) and by Overman *et al.* for Holsteins (O<sub>1</sub>-O<sub>2</sub>) and for Jerseys (O<sub>2</sub>-O<sub>3</sub>) have been included. Spot values are shown for herd milks reported by Davis *et al.* (8) for Holsteins (T<sub>1</sub>), Guernseys

(T<sub>2</sub>), Jerseys (T<sub>3</sub>) and Mixed (T<sub>m</sub>). Also shown are spot values for Holstein (I<sub>1</sub>) and Jersey (I<sub>2</sub>) milks reported by Shaw and Fourt (25), for 74,000 samples of producers' milks received by San Francisco during the period 1939-1947 (SF) as reported by Geiger (2) and for Holstein milk from herd F, table 3.

TABLE 1

*Average values for fat and solids-not-fat for milks grouped according to fat contents and screened for mastitis*

Range of fat	No. of samples	Fat (av.)	(Av.) Solids-not-fat
(%)		(%)	(%)
2.50-3.15			
Neg.	72	2.98	8.31
Pos.	26 (26.5%)	2.91	7.86
Av.*	98	2.96	8.19
3.00-3.50			
Neg.	157	3.28	8.40
Pos.	52 (24.9%)	3.30	8.11
Av.	209	3.29	8.33
3.60-4.20			
Neg.	136	3.89	8.82
Pos.	62 (31.3%)	3.79	8.21
Av.	198	3.86	8.63
4.00-4.30			
Neg.	39	4.15	9.01
Pos.	19 (32.8%)	4.14	8.33
Av.	58	4.15	8.79
4.25-5.00			
Neg.	96	4.62	9.23
Pos.	34 (26.2%)	4.65	9.00
Av.	130	4.63	9.17
5.10-5.80			
Neg.	54	5.48	9.50
Pos.	18 (25.0%)	5.32	9.39
Av.	72	5.44	9.47
5.75-6.25			
Neg.	35	6.00	9.72
Pos.	9 (20.5%)	6.02	9.44
Av.	44	6.01	9.70
6.00-8.00			
Neg.	56	6.60	9.83
Pos.	12 (17.6%)	6.74	9.45
Av.	68	6.62	9.76
Holsteins (over-all)			
Neg.	361	3.61	8.58
Pos.	161 (30.8%)	3.54	8.15
Av.	522	3.59	8.45
Jerseys (over-all)			
Neg.	179	5.50	9.55
Pos.	54 (23.2%)	5.50	9.44
Av.	233	5.50	9.53
Holsteins (over-all, not screened for mastitis)			
Av.	717	3.63	8.44
Jerseys (over-all, not screened)			
Av.	231	5.55	9.54
Ayrshires (not screened)			
Av.	100	3.96	8.74

\* All average values were weighted according to the no. of samples.

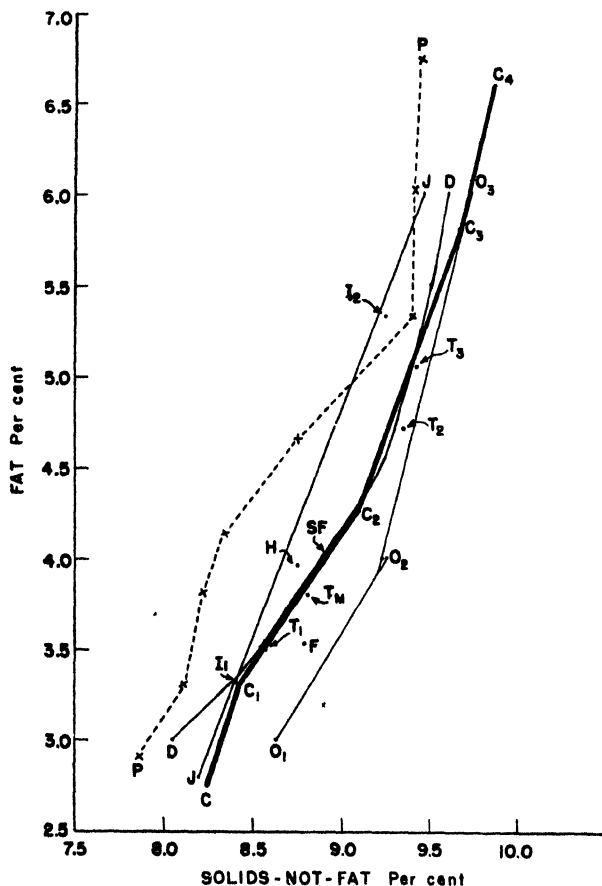


FIG. 2. A comparison of the relationship between fat and solids-not-fat for normal milks (table 1) with some representative published values.

*Legend for Fig. 2*

- Curve D-D —from median values (5)  
 J-J —per cent solids-not-fat = 0.4% fat + 7.07. (12)  
 C-C<sub>1</sub> " " " " " = 0.3151% fat + 7.3672. (table 2)  
 C<sub>1</sub>-C<sub>2</sub> " " " " " = 0.7% fat + 6.1. (table 2)  
 C<sub>2</sub>-C<sub>4</sub> " " " " " = 0.3846% fat + 7.44. (table 2)  
 C<sub>4</sub>-C<sub>6</sub> " " " " " = 0.2457% fat + 8.2340. (21)  
 O<sub>1</sub>-O<sub>2</sub> " " " " " = 0.6138% fat + 6.7917. (21)  
 O<sub>2</sub>-O<sub>4</sub> —same as C<sub>4</sub>-C<sub>6</sub> (Jersey) (21)  
 P-P —for milk from mastitis-positive cows  
 I<sub>1</sub>, I<sub>2</sub> —average for Holstein and Jersey milks (25)  
 T<sub>1</sub>, T<sub>2</sub>,  
 T<sub>3</sub>, T<sub>4</sub> —average for Holstein, Guernsey, Jersey and mixed herds (8)  
 F —an inbred Holstein herd  
 SF —average for over 74,000 samples of producers' milks (2)  
 H —average for 100 samples of Ayrshire milk

It is apparent that although the solids-not-fat increase with increasing fat concentrations, the relationship is not linear except for fairly narrow fat limits. This is in harmony with the views of others (4, 26). In the milk from mastitis-positive cows the proportion of solids-not-fat to fat is lower than in normal milks. For low fat milks these data for solids-not-fat agree with those reported by Jacobson but are somewhat higher than those arranged by Lythgoe, as reported by Brown and Ekroth. For milks above 3.3 per cent fat, the Jacobson values appear too low. The Overman *et al.* values for solids-not-fat for milks below 4.5 per cent fat are higher than for the milks of this study. As the milks increase in fat, agreement increases until the Overman equation for Jerseys is in complete agreement. The relationships shown by curve C-C<sub>4</sub> agree with those of Brown and Ekroth (5) and, therefore, with (17) (see fig. 1) in the range of 3.3 to 5.25 per cent fat, with those of Davis *et al.* (8), of Shaw and Fourt (25) for Holsteins and with those reported by Geiger (2). The lack of agreement of  $I_2$  possibly may be due to the high incidence of mastitis reported for this herd. The position of  $F$  may suggest that heredity is responsible for the high values of the single herds of Holsteins studied by Overman *et al.* (21) and by Kahlenberg and Voris (13) (see fig. 1).

That the relationship between fat and solids-not-fat shown by curve C-C<sub>4</sub>, fig. 2, may be considered to apply generally is given further confirmation by the agreement with the results reported by Nicholson and Lesser (20) for 4,816 samples of Friesian milk, by Bakalor (3) for 2,808 samples of herd milk, by Cranfield *et al.* (7) for 131 samples of Friesian herd milk, by White and Judkins (29) for Holstein and Ayrshire milks, by Tocher (26) for 676 samples of Ayrshire milk and by Lampert (15) for computed average values. It should be pointed out, however, that this relationship disagrees with those reported by Haecker (9), whose average values conform to those reported by Jacobson (12), by White and Judkins (29) for Jersey and Guernsey milks and by New York State (1) for genuine milks from "suspected" herds.

To further test the accuracy of the C-C<sub>4</sub> curve (fig. 2), weighted average values of fat and solids-not-fat were calculated for 1,616 samples of Holstein milk and for 916 samples of Jersey milk, collected under supervision and representing mostly 1- or 3-day composites, from the Arizona (8), Idaho (25) and the California cows. The ratio of fat to solids-not-fat of 3.5 to 8.47 for the Holsteins and of 5.29 to 9.38 for the Jerseys in each case is higher than that shown in the curve for normal milk. The values for solids-not-fat do correspond closely to the values calculated from the appropriate equations for abnormal milks (table 2). It may be assumed that at least 20 per cent of the cows were mastitis positive (28).

The equations of table 2 for the four segments of the curve C-C<sub>4</sub> of fig. 2 and for a similar curve drawn to represent the average values (table 1) serve to calculate the solids-not-fat, and therefore the total solids, of normal milks and those from cows 20-25 per cent of which are mastitis positive. The latter equations may be of greater practical significance in view of the high national incidence of mastitis of at least 20 per cent (28). It would be presumptuous, how-

ever, to believe that calculations can replace chemical determinations, or that the percentage of mastitis-positive cows in a herd can be calculated from the fat and solids-not-fat relationship. Equations may be a useful tool for the routine

TABLE 2

*Equations showing relationship of fat and solids-not-fat for various intervals of fat content*

Fat range	Equation
(%)	
	<i>For normal milk</i>
2.75-3.30	Per cent solids-not-fat = 0.3151% Fat + 7.3672
3.30-4.25	Per cent solids-not-fat = 0.70% Fat + 6.10
4.25-5.85	Per cent solids-not-fat = 0.3846% Fat + 7.44
5.90-6.75	Per cent solids-not-fat = 0.2457% Fat + 8.2340
	<i>For abnormal milk—20 to 25% of cows mastitis positive</i>
2.75-3.30	Per cent solids-not-fat = 0.35% fat + 7.18
3.30-4.25	Per cent solids-not-fat = 0.7041% Fat + 6.0056
4.25-6.00	Per cent solids-not-fat = 0.4007% fat + 7.2936
6.00-6.75	Per cent solids-not-fat = 0.0959% fat + 9.1248

predicting of the composition of producers' milk; they cannot be used for milk standardized by either the removal of cream or the addition of separated milk, unless the fat contents of the original milks are known (23). Blended milk also may be irregular.

THE INFLUENCE OF HEREDITARY FACTORS IN DETERMINING  
THE RELATIONSHIP OF FAT AND SOLIDS-NOT-FAT

Table 3 shows the results of the analyses of the milks of daughters of indi-

TABLE 3

*Analyses of the milk of daughters of individual sires*

Herd	Sire	No. of daughters	No. of samples	Fat	S.N.F.	Herd av.	
						Fat	S.N.F.
				(%)	(%)	(%)	(%)
D	1	12	14	3.50	8.61 (8.55) <sup>a</sup>	3.42	8.50 (8.49) <sup>a</sup>
	2	6	7	3.61	8.56 (8.63) <sup>a</sup>	(36 samples)	
	3	3	5	3.39	8.68 (8.47) <sup>a</sup>		
F	4	18	42	3.68	8.97 (8.68) <sup>a</sup>	3.53	8.77 (8.57) <sup>a</sup>
	5	11	22	3.31	8.55 (8.42) <sup>a</sup>	(93 samples)	
N	6	15	31	3.60	8.55 (8.62) <sup>a</sup>	3.63	8.47 (8.64) <sup>a</sup>
	7	13	27	3.45	8.67 (8.51) <sup>a</sup>	(157 samples)	
	8	5	10	3.50	8.77 (8.55) <sup>a</sup>		
	9	6	13	4.16	8.61 (9.01) <sup>a</sup>		
M	10	24	42	3.67	8.29 (8.55) <sup>a</sup>	3.78	8.47 (8.67) <sup>a</sup>
	11	24	37	3.87	8.59 (8.73) <sup>a</sup>	(157 samples)	
	12	17	32	3.56	8.43 (8.51) <sup>a</sup>		
	13	15	27	3.98	8.49 (8.81) <sup>a</sup>		

<sup>a</sup> The values within brackets, calculated from the appropriate equation (table 2), are included for comparison of the observed with the expected values.

vidual sires in four purebred herds. All the daughters were mastitis-negative with the exception of herd *M* which, as a basis for calculation, is assumed to have been 20 per cent positive.

The daughters of sires 1, 3, 4, 5, 7 and 8 produce milk with solids-not-fat higher than the values calculated from the appropriate equation in table 2. The milks of all of these daughters, with the exception of those of sire 5, are higher in solids-not-fat than their herd average, and also are higher than the values calculated from the average fat for the herd. It would appear that the incidence of mastitis in herd *M* was higher than the assumed 20 per cent.

Special attention is called to herd *F* (fig. 2). This herd is rather highly inbred and, at the time of sampling, was practically mastitis free. Sire 4 of this herd, and also sire 3 of herd *D*, transmit solids-not-fat relative to fat approaching the values found by Overman *et al.* (21) and Kahlenberg and Voris (13), both of whom reported on samples from single herds.

While the data are too few to establish that the secretion of solids-not-fat is inherited separately from that of the fat, they do lend support to the findings of others previously mentioned, and to the assertion by Van Rensburg (27), as a result of studies with a mastitis-free experimental herd, that the persistent secretion of milk low in non-fat solids is of hereditary origin.

#### SUMMARY AND CONCLUSIONS

A fairly comprehensive survey of the literature dealing with the relationship between the fat and of solids-not-fat contents of milks from cows within breeds and among breeds has revealed a great lack of agreement. This latter involves differences as to absolute values, but of equal or greater significance are the opposing views with respect to the differential increases of the solids-not-fat for a stated fat increment. Some of the regression equations designed to express these relationships are shown in fig. 1, in which two schools of thought, the linear and the non-linear, are exemplified.

The data presented were obtained by analysing for fat and solids-not-fat 717 samples of milk from five herds of purebred Holstein, 100 samples from two herds of purebred Ayrshire and 231 samples from one herd of purebred Jersey cows. Of these, 157 samples of Holstein and all samples of Jersey milk were 3-day composites. The collection of all samples was supervised. Of 522 samples of Holstein milks and 233 samples of Jersey milks, 20.8 and 23.2 per cent, respectively, were from mastitis-positive cows. These data have been presented in table 1 and those representing normal milk are presented in fig. 2 by fat increments in juxtaposition to representative published data.

The relationship between fat and solids-not-fat in both normal and abnormal milks does not appear to be linear except within restricted limits of fat percentage. No consistent relationship exists for milk from mastitis-positive cows. It already has been pointed out that the results agree very favorably with those of many workers, but are higher than those reported by Jacobson (12) except for low fat milks. They are lower than those reported by Overman *et al.* (21), especially in the normal fat range for Holstein milk.



Equations for calculating the solids-not-fat percentage from the fat percentage, within practical limits of fat concentration, have been established from the data and are shown in table 2. A separate set of equations for milks from herds containing 20 to 25 per cent mastitis-positive cows also is shown.

Analyses of the milks of mastitis-negative daughters of nine bulls support the belief that an hereditary factor is involved in the secretion of non-fat solids. A liberal interpretation of the data showing the role of the sire in transmitting high or low solids indicates that it may be possible to influence the compositional quality of milk by the use of sires "proven" with respect to solids-not-fat. By choosing bulls shown to transmit the factor for high solids-not-fat values, and at the same time limiting the ravages of mastitis, it should be possible to improve the compositional quality of the milk supply. Additional carefully obtained data are required to establish the significance of these results. It is suggested that this might be given consideration when organizing an artificial insemination program, as well as when selecting the herd sire. It also is suggested that the factor of inheritance, coupled with a varying incidence of mastitis, may be responsible for the divergence in the regression equations erected from data obtained from individual herds. This may account for the unusually high quality of the Holstein milk studied by Overman *et al.* (21).

To avoid additional confusion arising from studies of this nature it is recommended that: (1) care be exercised in securing representative and genuine samples at the source; (2) the cows be tested for udder abnormalities; (3) the milk of cows physiologically disturbed not be used; (4) the inheritance factor be given additional study; (5) regression equations be limited to comparatively narrow limits of fat contents.

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# INHERITED NON-LETHAL ANATOMICAL CHARACTERS IN CATTLE: A REVIEW<sup>1, 2</sup>

LESTER O. GILMORE

*Department of Dairy Husbandry, Ohio State University  
and Ohio Agricultural Experiment Station*

From the early realization that some families more than others consistently produced show-ring winners, the trend towards identifying the action of individual genes or groups of genes has been directed toward the herd-classification analysis of the different body parts and the relative influence of inheritance and environment in causing physical differences. The action of specific genes has been studied for certain characters.

When the over-all type is measured against the herd classification standard, a heritability estimate of approximately 30 per cent was found in Ayrshires by Tyler and Hyatt (100). The stability of type classification ratings, when measured by the repeatability with which individual officially recognized judges gave the same rating at a subsequent inspection, was found by Johnson and Lush (57) to be 0.34. The repeatability was 0.55 when a committee from the station made the ratings. The repeatability of individual inspectors, on consecutive ratings of the same cows, was found by Hyatt and Tyler (49) for 80 Ayrshire cows with an average of five type ratings to be 0.73, 0.82 and 0.62, respectively, for three different official inspectors. The correlation between the ratings given the same cows by different inspectors was 0.55. Of this group of cows, all of which had calved, 67.5 per cent had a range in ratings of one grade or less and 32.5 per cent ranged over three grades. High ratings were placed on cows if they were old or in the first 3 or last 2 mo. of their lactation. Hyatt *et al.* (50) tested the validity of requiring a female to calve before she is eligible to be classified. For 102 Ayrshires they compared the type ratings made by official inspectors before calving with the ratings made after calving. The per cent of animals varying zero, one, two and three grades, respectively, between ratings was 4.9, 51.0, 38.2 and 5.9. A low correlation appeared to exist between the ratings made at 6 and 18 mo., while the highest correlation existed between ratings made at either 18 or 24 mo. and those made at 30 mo. When the precalving ratings were divided into four groups from low to high, the group averages were in the same order when the postcalving records of the same animals were compared. However, the postcalving averages usually were closer to the general average.

With this progress there has been a slowly developing body of literature dealing with the role of individual genes in shaping body type.

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The material is presented according to the division of systematic anatomy primarily involved, i.e., skeleton, integument, sense organs, reproductive organs, muscle.

*Shape of skull.* Certain characteristics of the skull have been so definite that selection preference has fixed certain shapes and sizes of heads as breed characters. Thus, the preference in Jerseys (*Bos longifrons*) is for a double-dish which refers to an incurving between the eyes and to the angle at which the nasal bones set onto the frontal bones giving a pronounced stop. The work of Stockard (96) on dogs indicates this condition might be caused by an achondroplasia of the basiophenoid and basioccipital bones. This condition and a cranial crest are associated with a characteristic histological pattern of the thyroid and anterior hypophysis. The eye sockets are pronounced and the forehead is wide. The skulls of *Bos primigenus* are longer and flatter across the forehead with less prominent eye sockets than typical skulls of *Bos longifrons*.

In general, three pairs of words serve to characterize the heads within the breed and, therefore, to describe some characters, presumably inherited, in addition to those just reviewed: strong versus weak; refined versus coarse; and character versus lack of character (plain). While these terms are all relative, their meaning is definite enough to one familiar with cattle for them to have real value. Very little has been done to work out the number of genes involved in head characters and their specific effects. However, among persons familiar with cattle type, the head characters often are so pronounced that daughters of a particular bull or cow can be picked out on that basis. The work of Hurst reviewed by Gowen (40) and the observations of Cole and Johansson (17) indicate that the length of head is affected by different genes and that the long narrow (*primigenus*) head is dominant to the short head. The narrow width of Yak skulls is dominant to the wide skull of *Bos taurus* (1a).

*Prognathia* (undershot jaw). Cattle with the lower jaw projecting beyond the upper jaw have been referred to by Darwin in 1909 (27) as also having a short broad forehead. The inequality of the jaws was so great that it was impossible to close the "lips." The observations in sheep by Nordby *et al.* (80) and in dogs by Stockard (96), showed that prognathia can result from a normal upper jaw and abnormally long lower jaw; a short upper jaw, associated with a short face or skull base, with a normal lower jaw; or to a short upper jaw and an exaggerated lower jaw. Liddell (65) showed that thyroidectomy caused shortened nasal and frontal bones, but had no effect on mandibular growth. Cattle in this country answering the same general description as those observed by Darwin, but less extreme, have been observed by Becker (7) in grade Jerseys and the reviewer in a grade Holstein. The observations by Becker indicate that the inheritance involved a single recessive autosomal gene.

*Brachygnathia* (parrot mouth, overshot jaw, pig jaw, bird face, micrognathia). In some cattle, the lower jaw is shorter than the upper. Brachygnathia has been observed by the reviewer in Guernsey, Holstein and Jersey cattle, although it may exist also in other breeds. Limited observation in cattle and the sheep studies of Nordby *et al.* (80) indicated an inherited basis but that it is caused by several genes, some of which are dominant and some recessive.

To the various types of subnormal development of the head (including brachygnathia), Wright (111) applied the term otocephaly. The different otocephalic monsters arising in certain lines of his guinea pig stock represented the activity of genes taking place in cells of different stages of development. Using cases from both cattle and sheep, parallelisms have been noted for six of the 17 degrees of severity observed in guinea pigs as follows: (a) Slight to considerable (3 in.) reduction in lower jaw in cattle by Gilmore (35) and in sheep by Nordby *et al.* (80). (b) Mandible lacking (agnathia) observed in cattle by Ely *et al.* (28). (c) Jaws abnormal, external ear openings and eyes lacking as in a Holstein-Friesian calf carried over term with malformation of lower jaw, snout-like muzzle, and nostrils not patent observed by Gilmore (35). (d) Fused nostrils; Illancic (54) observed this same character in cattle. (e) Headless except for single small median external ear. A faceless lamb was reported by Winters and Kernkamp (106) and a headless lamb by Fasten (30). Recessive genes have been suggested for (b) and (e), with a dominant lethal inhibited by a recessive gene postulated by Krallinger (63) for (d).

*Horns.* The exceptional cases in which horned or scurred offspring result from the mating of horned cattle with those presumed to be homozygous for the polled character and the sex difference in the appearance of horns from horned parents as noted by Churchill (14), brought out the need for an interpretation more adequate than a single allelic pair. The interest in breeding polled cattle has been increasing. Registered herds of polled Ayrshire, Guernsey, Hereford, Holstein-Friesian, Jersey and Shorthorn cattle exist, in addition to those of the established naturally-occurring hornless breeds. Apparently, polled Brown Swiss are rare. In some breeds the registration is separately maintained for polled cattle. The current interest in polled cattle is indicated by the formation in February, 1949, of the National Polled Cattle Club, which was formed to develop interest in hornless cattle (26).

The horns of cattle are sheathed as contrasted by Auld (4) to animals with solid horns, either persistent (giraffe) or deciduous (deer) and to hornless species of several genera including the camel, llama, alpaca, guanaco, chevrotain, water and musk deer. Cattle share the peculiarities of sheathed horns with antelope, goats and sheep. The complexity of horn inheritance is shown by the fact that in sheep, Ibsen (51) required eight different pairs of alleles to explain all conditions of horns, scurs and hornlessness reviewed by him.

To explain the results of recorded matings with reference to horns in cattle, White and Ibsen (105) postulated the presence of four pairs of alleles. The dominant gene *H* is assumed to be present homozygously in all cattle. The symbol *P* is retained to denote the gene for polled. It is completely dominant to its allele *p* and completely epistatic to *H*.

Next is postulated a second gene for horns, *Ha*, the *a* referring to Africa, but logically it also might refer to Ayrshire. In addition to being found in the Ayrshire breed, it is found in other horned breeds such as the West Highland, Hereford, Holstein-Friesian, Guernsey and Jersey. Its incidence in the dairy breeds seems to be low. The presence of this gene in these breeds became evident when cows produced some horned sons to the service of Angus or Red Polled bulls.

The gene *Ha* is epistatic to *P* in the bull. An *HH*, *Pp*, *Haha* heifer is polled, but the relation of *HaHa* to *P* remains undetermined. The relationship of *H* to *Ha* is not known. Ibsen (52) assumed close linkage between *P* and *ha* to account for the lack of horned individuals appearing in the Aberdeen-Angus breed.

The fourth gene postulated by White and Ibsen is responsible for scurs when dominant, i.e., *Sc*. As compared to *Ha*, a relatively large number of cattle in the horned dairy breeds carry *Sc*. *Sc* has the same relationship to *P* as does *Ha*. However, in horned animals, *pp*, *HH* is epistatic to *Sc*. That sex modifies the expression of one or more of the genes involved is verified by Cole and Johansson (17) who found it questionable if any  $F_1$  steers were clean polled in their crosses between Angus and either Jerseys or Holstein-Friesians.

Further explanation is needed to account for the long horn of the Ayrshire, Hereford and Highland cattle and for other breed differences not noted above. Limited observations indicate horn length to be intermediate in various crosses with Ayrshires (36). Black (9) observed scurred or horned sons in all cases and horned females in three-fourths of the offspring of Angus  $\times$  Indian cattle crosses. In Holstein-Friesians, Jerseys and Shorthorns, the horns are incurving, while in Brown Swiss, the horns curve slightly upward. Indian breeds and water buffalo present still other horn characteristics. In crosses between Indian cattle and Yak, Zawadowsky (114) considered at least two pair of alleles involved. Evidence is cited by the above authors for believing that early cattle were polled and that the original mutation was *P* to *p*. For polled animals to have appeared spontaneously in most horned breeds fairly recently, this reverse mutation, *p* to *P*, must occur rather frequently. The frequency of this mutation has been estimated at 1:20,000 by White and Ibsen (105).

Two centers of ossification in true horns were found by Dove (25), one in the derma and the other in the periosteum of the skull at the point where the horn is located. The bony core (os cornu) arises from the first of these centers. In the early stages of development, this bud fuses through the frontal periosteum to the frontal bone with an accompanying formation of a supporting boss. If fusion does not take place, a scur results which is movable with the skin. The scur is considered by Dove usually to contain a horn core. Whether the small size of the horn core in scurs is due to a less adequate blood and nerve supply than would be the case if it fused to the boss in normal horn formation, appears not to have been determined. However, Wislocki and Singer (107) found that deer antlers denervated just before growth started became dwarfed and deformed as compared to the normal antler.

The action of all four specific genes postulated by White and Ibsen (105) has not been entirely reconciled with the histological findings of Dove (25). However, the latter concluded that the presence of horns, *pp*, is controlled by a single factor, the horn core, which also causes the development of the horn sheath or shell. The polled condition, *P*, therefore, would be the inhibition of horn core formation. There remains to be explained the relationship of *Sc*, *H* and *Ha* to the formation of the boss and to the ossification process. That the boss may occur without horns has been observed by Dove in Jersey or Holstein  $\times$

Angus crossbreds. The reviewer has observed them also on naturally polled Ayrshires. Dove suggested that the frontal eminence (poll) of horned and polled breeds differed, not because of gene interaction, but because of the secondary action of the genes causing (permitting) horns that cause a physical accommodation of neighboring parts. The work of Curson, according to Cole and Johansson (17), indicated that debudding young calves causes a pronounced change in the shape of the skull. However, from pictures of animals published by the respective breed associations, both the gothic (high) and Roman (flat) poll can be observed on both Red Poll and Aberdeen-Angus cattle, whereas, Galloway cattle are characterized by their flat poll. Auld (5) showed pictures of both kinds of polls in hornless skulls. In the horned breeds, Ayrshire and Shorthorn have a flatter poll than Holstein-Friesian and Jerseys.

*Feet and legs.* That there are breed differences in straightness of legs is indicated by Brown Swiss and Shorthorn, generally conceded to be the straightest. The observed differences within breeds also lend much support to the importance of inherited influences, but the exact extent and nature of the genetic influences are not known. The present tendency is to increase the emphasis on feet and legs up to eight points out of 100 on the judging score card.

The feet and legs are considered to include all of the parts of the appendicular skeleton anatomically considered as fore and rear limbs. The fore or thoracic limb consists of the shoulder girdle, the arm or humerus, the forearm (radius and ulna) and the manus ("foot"). The manus consists of the carpus, metacarpus and phalanges. The rear or pelvic limb consists also of four parts, the pelvic girdle (ox coxae), thigh (femur), leg and "foot" (pes). The pes consists of the tarsus, metatarsus and phalanges (92).

Some general defects resulting from poor care generally are recognized. Keeping bulls in muddy pens is associated with long, turned up hoofs and weakened pasterns. Standing platforms have been associated with faulty legs in cows. In both cows and bulls, toeing out in front has been considered to be caused by grazing or pulling in hilly or uneven ground. This abnormality is assumed to develop in response to the animal turning its legs to distribute the body weight more uniformly on the bottom of the hoof.

*Toeing out.* The divergence of the cleft between the toes from a line parallel to the body has been used to measure the degree of severity. Cases affected so severely as to show a toeing out of 70 degrees have been reported by Kappeli (62). In this study the legs were considered normal unless the angle was more than 15 degrees.

In spite of possible non-inherited influences, there appears to be some definite inherited cause for this condition. While it is present at an early age, it develops still further with advancing age. It is associated with a narrow chest floor. In Switzerland, toeing out appeared more in Simmental cattle than in cattle of other breeds grazing on similar mountain pasture. In a case of close observations on animals confined to even meadows, severe toeing out was found to be transmitted by the bull, Zar (40 degrees), through both a son (70 degrees) and a daughter (40 degrees) to a double grandson (70 degrees).



*Short leggedness* (duck-legged, compact). The short-legged Herefords found in Texas by Lush (68) were not distinguishable from normal Herefords except for the short legs. There was no dwarfism of other parts of the body. Only the long bones of the limbs (metacarpus, radius-ulna and humerus of the fore limb and metatarsus, tibia and femur of the hind limb) appear to be affected. The Shorthorns described by Stonaker and Tom (97) in Colorado, Nebraska and Kansas having this or a similar character were compact and thick in the neck and in body fleshing. Whether or not these breed differences are due to modifiers, the variable expression of the gene or to differences in describing observations remains unknown. Short leggedness can be identified at birth and is distinct throughout the life of the animal. In balance trials and carcass analyses by Washburn *et al.* (103) short-legged or compact Shorthorns were found to be less efficient during growth than the normal in the utilization of digested dry matter. The compact type required 70 days less time in the fattening lot to reach a finished condition. The carcasses of the normal steers were relatively light in bone and carried more fat in the carcass cuts. Short-leggedness is inherited as a single autosomal dominant.

*Proportionate dwarfism.* Although the birth weight, height at withers and heart girth average somewhat less at birth than do normal calves, dwarfism cannot be detected at birth because individual calves lie within the normal range. At 12 to 14 mo. of age, however, the differences are great enough to permit diagnosing whether the calf is dwarf or normal. As the name indicates, all body parts are dwarfed in proportion to their size. This characteristic distinguishes it from short leggedness. Lactation in the dwarfs was normal in proportion to body size but reproduction was at a lower level. This character apparently parallels that of pituitary hypoplasia in goats (Epstein, 29) and dwarfism in mice (Smith and MacDowel, 95) in which latter case no acidophiles are found in the anterior lobe of the hypophysis. DeBeer and Gruneberg (21) found no acidophiles in dwarf mice at 7 days, although they did not stop growing until 17 to 21 days. Due to the production of dwarfs from related parents and because they were uniform with no intermediate types, the character appears to be due to a single gene on one of the autosomes with complete dominance of the allele. In cattle, this type of dwarfism was found in the University of California inbreeding experiment with Jerseys by Mead *et al.* (73).

*Flexed pasterns.* This involvement of the ligaments of the pastern (phalangeal bones) causes an afflicted calf to stand on its toes during the first few days to a week of its post-natal life. In severe cases, the toes are turned completely under. The fore pasterns are always involved and frequently also the rear pasterns. The autosomal recessive gene responsible for flexed pasterns was found in Jerseys in California by Mead *et al.* (74). The same characteristics have been observed in a Gir bull calf by Khare (59).

*Bowed pasterns.* The affected pasterns are found on the rear legs. No cases are known in which the pastern was dissected to show the exact nature of abnormality. The pastern is incurving as though one or more of the lateral phalanges were longer than the corresponding medial phalange. There is much variation

in the extent to which different affected pasterns are bowed. This abnormality was found in both Jerseys and Holstein-Friesians by Atkeson *et al.* (2), but the mode of inheritance is not known.

**Sickle hocks.** This abnormality refers to the angle at which the tibia and metatarsus are set at the point of the tarsus or hock. The approved show standard requires a slight set or angle and discriminates against legs that are too straight, or posty and those that are set at such an angle that the metatarsus, or cannon bone, extends far under the belly, giving a rounded or sickle appearance from a side view. There is a wide variation between individuals and some difference between breeds.

Some difference has been noted in the ability of cattle to withstand concrete platforms, with preference given to the breeds with straighter legs (Clapp, 15). Cases were noted in which the cow's rear legs were so sickled that difficulty was encountered in natural breeding. Sickle hocks are especially objectionable where combined in the same animal with toeing out and weak pasterns. In horses, Wriedt (110) pointed out that sickle hocks was caused by a relatively long femur, although this appears not to be the obvious case in cattle (35).

In Ayrshires, Habel (43) found that 244 cows of varying ages from 2 to 16 yr. had an average inclination at the hock of 78.7 degrees from the horizontal plane. No correlation was found between unrelated members in the same herd, thereby indicating environment was not the cause. On the other hand, a correlation of 0.41 existed between 37 daughters and their dams. It was concluded that the amount of metatarsal inclination is affected by multiple genes, some of which are thought by Ibsen (53) to be dominant.

**Polydactylism.** Extra toes were found in a family of Holstein-Friesians in Illinois by Roberts (89). A cow, her daughter and three grandsons each had three toes on the fore legs. One of the grandsons had four toes on one rear leg and five on the other. The other four affected animals each had three toes on the rear legs. Severson (91) reported a case in which an extra digit extended from the splint or cannon bone. The cannon bone is normal with a fusion of the third and fourth metacarpal (metatarsal) bones. The first, second and/or fifth metacarpal (metatarsal) bones apparently are not always prevented from developing. The corresponding phalanges, or toes, also are present. The evidence presented indicates dominant inheritance for the presence of extra digits. This would indicate that the original mutation to the "normal" number of two digits was a recessive and that the reverse mutation also occurs. Polydactylism also occurs in poultry and cats (Danforth, 20), guinea pigs (Wright, 112) and man (Pipkin and Pipkin, 84), where the expression is variable. Sometimes it behaves as if it were due to a dominant gene. A sex-linked recessive gene for polydactylism in mice, that expresses itself with about 25 per cent penetrance, was reported by Chase (13).

Polydactylism in cattle has been reported by Morrill (76). An extra toe appeared on each front foot. The affected calves were able to function normally at first but became tender and lame as they approach a weight of 600 to 800 lb. The appearance of the abnormality among males only of one Hereford cow fam-

ily suggested to this investigator that sex-linkage might be involved (91a).

*Misshappen feet.* A digital anomaly involving a spreading of the hoof phalanges was concluded by Mead *et al.* (74a) to result from faulty muscles and ligaments. Affected calves became lame from 2 to 4 mo. of age and became progressively worse until at 18 mo. obvious discomfort was caused by standing or walking. The appearance of this character in a herd of registered Jerseys was concluded to be conditioned by a single autosomal gene.

*Angle of rib.* Smith (94) cited the work of Duerst, who studied the angle of the last rib with the horizontal. In several strains of cattle a close relation existed between the angle of rib possessed by daughters and their sires and by sons and their respective dams. This suggested sex-linked inheritance has been checked by several investigators according to Smith, but they have not reached a unanimous conclusion.

*Depth of body.* That depth of body is inherited is indicated by the results of the crossbreeding work at Maine as reported by Gowen (39). Cattle of several dairy breeds (Guernsey, Holstein-Friesian and Jersey) were crossed with Aberdeen-Angus and gave offspring resembling more the dairy parent with respect to the middle. The depth through the middle generally is considered to be affected by factors other than those affecting depths through the region of the heart. Whether or not there are different factors influencing the depth of the middle has not been determined.

*Loin characteristics.* The desired loin is long, wide, thin and level. The European breeds from which most cattle in this country originated possessed six lumbar vertebrae in common with the Indian breeds. Some relatives of the domestic cow (Banting) have five lumbar vertebrae (Goodrich, 38), a fact that suggests a mutation in the number of vertebrae for this region has occurred. Whether or not there have been other mutations at this gene-locus is not known. Very few observations appear to have been made in the dairy breeds with this in mind.

The loin sometimes is roached or arched (35). More frequently, however, cattle are low in the loin. While an inherited tendency appears to be responsible, the nature of these structural characters is unknown.

*Rump.* Few single parts of the bovine anatomy have attracted the fancy of dairy cattle breeders as much as the rump, which preferably is long and wide. The top line should be level and the tail head neatly attached. The levelness from hooks to pins apparently differs between breeds. The thurl should be wide and full. Presumably, each of the characteristics is governed by different genes. The European breeds have five sacral vertebrae while the Indian cattle have four (38, 4). Whether or not similar differences exist within each species and breed appears to be unknown. The lack of a level rump has been particularly discriminated against in selection, partly because it was considered unsightly and partly because it was thought to be correlated with an inclined udder floor. The latter has been found by Leighton *et al.* (64) not to be the case. Neither is there any correlation with total milk production. The sloping of the rump is

least up to 5 mo. and increases progressively up to 5 yr. The inheritance is thought to be that of a quantitative character. It is found in all breeds.

**Wrytail.** This malformation of the sacral vertebrae appears upon casual inspection to consist of a distorted tailhead that sets at an angle laterally to the vertebral column. The degree of distortion varies greatly, with cases approximating 45 degrees having been observed. By the use of X-ray analysis it has been found by Gilmore and Sellers (37) that the declination is caused by longer growth on one side of the body of sacral vertebra, along with a greater inter-vertebral distance than that occurring on the other side. This malformation has been seen in young heifers but no cases of its being present at birth have been reported. The presence of wrytail was found by Atkeson *et al.* (3) to be conditioned by an autosomal recessive gene. It is found in the Jersey, Brown Swiss (20 per cent of 505 animals in 34 randomly selected herds), Guernsey, Holstein (20-30 per cent), Ayrshire and Red Polled breeds. If it occurs in the beef breeds, it apparently has not been detected or it occurs less frequently than in the dairy breeds.

**Screwtail.** Screwtail resembles wrytail except that it is more severe and occurs in the posterior coccygeal region. There is a fusion of one or more pairs of adjacent vertebrae that involve a twisting as well as shortening of the vertebrae. Therefore, while it is apparent at birth, the fusion may not be completed for several weeks thereafter. No cases in cattle have been reported to involve more than two pairs of vertebra. In mice affected by a similar (or identical) condition, the beginning of the abnormality was noted in the fourteenth day of gestation. The tissue that is to develop into the disks between the vertebrae fails to form normal fibrous tissue on the side of the kink. MacDowell *et al.* (69) found the pleiotropic effects of the responsible gene in mice to include the pelvis, vertebrae anterior to the coccygeal region, sternum and head parts. The gene causing screwtail is inherited as a recessive and has been found in inbred Red Polled cattle by Knapp *et al.* (61) in Florida. It also has been observed in an inbred beef Shorthorn cow and her inbred son in Minnesota. Furthermore, screwtail has been observed in Holstein-Friesian and Jersey cows but no evidence that it is inherited in the last two breeds is known to have been produced. The same condition has been found in swine by Nordby (79), in different species of mice by Huestis and Barto (48) and in dogs by Stockard (96), where it also is inherited as a recessive. In dogs the allele of the gene for screwtail did not exhibit complete dominance.

**Vestigial tail.** Sometimes calves are born with only 4 to 6 in. of tail, corresponding approximately to six coccygeal vertebrae. The tail stub may curl to one side. Other abnormalities may include a malformed loin. Information on the existence of this abnormality has been obtained from Norton (81) and observations of different calves by Brandt (10), Salisbury (90), Green and Fenstermacher (41) and the reviewer. All known cases were Holstein-Friesians, Shorthorns, Angus or calves of mixed breeding. Salisbury observed several cases that resulted from using a bull on his paternal half sisters. It may be concluded tentatively that a recessive gene is responsible.

*Notched ear 1.* The first ear character of this name was described by Yamane (113) in Japanese Ayrshire cattle imported from the United States and in a California herd from which foundation cows were exported to Japan. The notch or nick occurs symmetrically in both ears at the distal extremity. Incomplete dominance of a single autosomal gene is the suggested mode of inheritance. In the homozygous dominant condition, the ear is reduced to one half its normal size, while in the heterozygous condition, the reduction is slight. In all but one case of the five cows and eight bulls in volumes I and II of the American Ayrshire Record (1876 and 1878), the affected animals trace back to the bull Eglinton, imported in 1859 from Scotland to the United States. This bull sired 21 calves with notched ears. The affected Japanese Ayrshires traced back to Express 4503, purchased in California. Express descended from Eglinton and sired 35 offspring, all of which were notch eared. A similar form of notched ear in Ohio Ayrshires has been found by Heizer (Snyder, 98).

*Notched ear 2.* This is similar to the heterozygote of the first type. Both ears are always the same with a length of 5 in. compared to a normal ear of 7 in. The heterozygote varies from short to normal. This type of notched ear is found in Norwegian cattle (Wriedt, 108).

*Notched ear 3.* The third pattern of notched ear has the missing part on the lower edge of the ear. Both ears may not be notched to the same extent. There is a doubling of the skin of the projection at the medial border with deep notches. This character was found in the descendants of two registered Jersey bulls in Texas by Lush (66). One notch-eared calf by an affected sire and from a cow with no Jersey breeding indicates the absence of interacting "Jersey" genes. It is inherited as a simple autosomal dominant.

*Double ears.* The fourth ear abnormality is the presence of a flat piece of ear tissue that grows on to the upper rear portion of the ear (Lush, 67). It extends from the base of the ear to a point about halfway from the head attachment to the outer edge. The doubling in this case should be distinguished from the doubling occurring in Jerseys (Notched 3), in which the doubled projection is in the under edge. Both ears are affected the same. All the information available indicates that the inheritance is due to a single dominant gene that traces back to a single bull imported in 1906 from the Krishna Valley, India.

*Drooping ears.* Incomplete evidence from crossing Indian breeds with European breeds in Texas, by Nabours (78), indicates the inheritance of the latter type to be dominant.

*Sweat glands of the ear.* Four types of sweat glands have been found in the auricle of the ear by Kalmykov (58) and Stakalic (95a). The possible significance of this finding rests with the positive correlation between production and the number of these sweat glands. The relationship was not altered with age and the glands were not well-developed in the bull.

*Muzzle pattern.* The first to suggest an inherited basis for muzzle or nose patterns in cattle appears to have been Boehme in 1910 (Gowen, 40). Petersen (82) investigated the use of muzzle prints for the positive identification of cows on official test. The belief in an inherited basis for this pattern rests largely

with the individuality of the animal with respect to a particular print. As with human fingerprints, no two cows have exactly the same print. It is characteristic throughout life. Identical twins have very similar patterns but marked differences are found when one pair of identical twins is compared to another. The muzzle is formed by the fiftieth day of embryonic life according to Habu (44). The nose pattern is clearly evident at 58 days. Muzzle patterns may be roughly grouped in the following order with the most frequently occurring patterns listed first:

(a) Median line extending from lower to upper edges of muzzle. Lines radiate from this line. Pattern may or may not be essentially symmetrical. (b) Median line extends from lower edge of muzzle part way to upper edge. Radiating lines give a Y-shaped pattern. (c) Radiating lines converge at a point near lower edge of muzzle giving a V pattern. (d) Irregular lines forming a greater complex than above. Some lines may run horizontally. The mode of inheritance is not known. Habu concluded that sex linkage was not involved.

*Semi-hairlessness.* This also is called hypotrichosis, which means subnormal hair development. This term, however, has been used to refer to a rather completely hairless lethal condition. Semi-hairlessness is not lethal, although affected animals do not grow as well as other animals. They are mild in temperament. The affected calves are born normal but are thin in flesh. The involved gene probably affects cysteine metabolism (Martin and Gardner, 71). As feeding this amino acid to congenital hairless rats caused a stimulating growth of the hair that lasted throughout a 30-day experiment, the cysteine was concluded to act through the sulfhydryl group to stimulate the hair follicle, thus resulting in a trichogenic action. The difference between the semi- and completely hairless condition has not been differentiated by this method. In addition to thinning out the hair, it also is curly. A recessive gene is responsible and was found in polled Herefords by Craft and Blizzard (19) in Oklahoma. A similar condition was found in Wisconsin Holstein-Friesians by Cole (16), except that affected animals approached the normal condition with age. Inherited semi-hairless conditions have been described also in the goat, sheep, pig, horse, dog, cat, rabbit, mouse and rat (Kislovsky, 60).

*Escutcheon.* Much attention has been given to this characteristic since Guenon (42a) associated it closely with milk yield by devising an elaborate system which involved dividing all cows according to size into large, medium and small. The escutcheons of each group were grouped into classes and orders. Numerous workers have studied further the relationship between the escutcheon patterns and such characters as body dimensions and milk yield (Brun, 11). Diverse results have been obtained. Brun found a positive relationship between escutcheon pattern and milk and fat yield, while Hooper (47) found no such relationship to exist. Most authors have classified the patterns into relatively few classes, however. That genes definitely influence the shape of the escutcheon is evident from the reported observations and from the extreme similarity found between identical twins as compared to the wide variation found between twin pairs noted by the reviewer.

*Curly hair.* At birth the hair in the Ayrshires described by Eldridge *et al.* (27a) was curly because of restrictions in the hair rather than because the hair was flat. Affected adults have a wooly appearance, like that of a newborn Karakul lamb. These workers agree with Johansson (55) who concluded from limited data on Swedish polled cattle that a dominant gene is responsible. Recessive curliness has been described in the Tuxer, Simmentaler and Pinzgauer breeds by Adametz (1). The type of curly hair in the Galloway was found by Pratt (86) to be recessive to the normally occurring straight hair of the Holstein-Friesian.

*Macrotrichosis.* The large hair or hairs that distinguish this character may be present in various locations on the mandible or maxilla, usually in an area more or less under the eye (Gilmore, 35). However, this also may be found underneath the lower jaw between the right and left mandible. A papilla varying in size up to a centimeter in length sometimes is associated with it. In no case yet observed did this papilla become "wattle like." In several cases it appeared on a calf without either parent having had it, thus suggesting recessive inheritance.

*Smooth tongue* (epitheliogenesis imperfecta lingua bovis). With many similarities to hereditary hypochromic anemia in man, the most striking feature in cattle is the smoothness of the tongue, as described by DeGroot (22, 23). The filiform papillae are normal in length but very thinly spaced. The mucous membrane is thickened. There is persistent salivation, eczema, alopecia, folding of skin and defective hair growth. The red blood cells are normal in number but abnormal in diameter. The iron content of the blood serum is low. This abnormality is widespread in certain lines of cattle in Holland in which 350 affected cattle of both sexes all trace back to one bull within 10 generations. It is conditioned by a single autosomal recessive gene.

*Congenital cataract.* At birth the eyes may be smaller than normal (Small, 93). The most reliable diagnostic character is the lens, which is opaque and reduced in early calfhood. With advancing age, the cornea (outer layer of anterior chamber) becomes distorted and enlarged. Affected animals are sensitive to strong light as noticed when they face the sun. Total blindness is unlikely, except in extreme cases.

Congenital cataract is inherited as a single autosomal recessive character. It has been found in Holstein-Friesians in Illinois by Detlefson and Yapp (24) and in California Jerseys by Gregory *et al.* (42).

*Strabismus* (cross eye). This second eye defect is not detectable at birth but is apparent after 6 mo. to 1 yr., with most cases being identified after 12 mo. The eyes are crossed and bulge or protrude abnormally. The condition gets progressively worse with age, vision being greatly impaired in some adult cows. The inheritance of strabismus behaves as other single autosomal recessive characters. It is found in both sexes and was brought to light by inbreeding. So far, it has been reported as occurring only in Jerseys in the California breeding project. However, other cases have been known in Jerseys that are thought to trace to a source that is different from the California herd (Regan *et al.*, 88).

*Night blindness.* Three Shorthorns in Oklahoma were found by Craft (18) to exhibit a type of vision that was defective at night with less serious effects in the bright moonlight. Presumably, the rods and cones of the retina were defective. Both sexes were involved and all traced in both parental lines to a common ancestor. Two were out of the same dam. If this abnormality is inherited, a single autosomal recessive gene is indicated.

*Udder.* Most phases of udder development are considered normal and as such have not received the attention from the standpoint of inheritance that certain undesirable characters have. Most of the undesirable characteristics of the udder thought to have an inherited basis have not been worked out in detail. It should be pointed out that between identical twins the similarity of udder size, shape and quality is so striking as to increase the belief that inheritance is a more important factor than ordinarily supposed (Hansson, 45).

*Udder slope (levelness).* The maximum slope has been found to be at 79 to 90 mo. of age by Leighton and Graves (64). Contrary to popular opinion, the slope of the udder was found not to be correlated to slope of rump. Neither was it related to total milk produced. The inheritance for sloping udder appears to be of a blending nature. It is found in all breeds.

*Teat sinus topography.* The mucous lining of the teat sinus may be smooth or with different numbers of pouches of varying sizes. This topography, which may not be related to the incidence of mastitis, was found by Murphy (77) to be characteristic of the breed and of the individual. The four teats of a cow, while showing some variation, usually were classified into the same group.

*Fused teats.* The udder is poorly shaped, possibly as a result of the fusion or partial fusion of the two teats on a side. In some instances both sides are involved. In the male the rudimentary teats are abnormal and are hardly perceptible. This maldevelopment has been reported in Guernseys in Ohio by Heizer (46) and Herefords in South Dakota by Johnson (56). Inbreeding had been followed in several of the herds in which it was found. The inheritance is that of a single autosomal recessive.

With further regard to the number of functional quarters, Black (9) lists udders with four quarters as dominant to udders with two quarters.

*Supernumerary teats.* That the presence of extra teats is based on the genotype is shown by the high frequency of occurrence in some herds of dairy cattle and the complete absence in others. Marked differences between the daughters by different bulls also has been observed and breed differences have been reviewed by Smith and Robison (94). They may be present between the normal teats, in which case they are called intercalary teats. The other two positions are to the rear of the normal teats and attached to them. Supernumerary teats are found as frequently on the right as on the left side and also may occur on both sides. Gifford (32) found supernumerary teats in 25.8 per cent of 4,831 females and 14 per cent of 135 males observed in Missouri dairy herds. The same worker (33) found no significant evidence that extra teats were associated with producing ability. They may be connected to extra functional glands. Magnusson (70) reported a case of a six-teated cow giving equal quantities of



milk from each of six teats. Supernumerary teats apparently are found in all breeds but the exact mode of inheritance is unknown. It has been suggested that recessive genes are responsible for the intercalary teats. A different autosomal gene probably is responsible for the after teats. It is thought to be dominant. In swine the number of teats is a typical case of quantitative inheritance without dominant genes being involved (Plum, 85).

*Testicular characteristics.* Although the literature on inherited characteristics causing deviations from the normal is indeed meager, a few statements are important.

In regard to the failure of one or both testes to descend, Barrett (6) noted what appears to be a case of a dominant mutation causing monorchidism. Cryptorchidism is known to be inherited in other species. McPhee and Buckley (72) concluded that most cases observed by them in Chester White swine could be accounted for by a recessive gene. Rea (87) pointed out that cryptorchidism is not unusual in humans, as it occurs once in every 500 males.

Carl (12) reported on two bulls by a common grandsire in which the left testis was smaller than the right and lay parallel, instead of perpendicular, to the body.

*Umbilical hernia.* Hernias are caused by the presence of abnormal openings through which other tissue can protrude. The umbilical ring is the opening through the abdominal wall that accommodates the umbilical stalk during fetal development. Its failure to close after birth may permit the omentum and intestine to protrude. The hernia may appear during the first month of post natal life and persist for varying lengths of time. They have been observed to persist up to 8 mo. in bulls and 18 mo. in females without receding. Calves closely related to affected animals have been observed in which the muscles of the umbilical ring did not close for several months, as detected by palpation, without evidence of hernia. The relation of hernia in bulls to reproductive inefficiency and other contributions to this problem has been reviewed by Gilmore (34). Umbilical hernia has been observed in both bulls and heifers. It may be inherited as an autosomal dominant with low penetrance (Warren and Atkeson, 101). A similar condition exists in rats where both sexes are affected. However, Moore and Schaible (75) concluded that several genes are involved as did Warwick (102) for swine and Phillips and Felton (83) for dogs.

*Inguinal hernia.* Although inguinal hernia occurs in cattle according to Frank (31) and Tuff (99), it occurs less frequently than in other farm animals. Its mode of inheritance is unknown.

*Bulging thigh.* Experimental evidence for the existence of inherited differences in the fullness of the thigh between dairy and beef breeds came from the crossbreeding studies at Maine (39). The incurving thigh of the dairy parent, whether it be caused by one or more genes, was dominant to the bulging thigh of the beef parent.

*Double muscle* (Yorkshire, doppellender, horse rump). Cattle expressing this character are abnormally thick and full in the thighs and loin due to the doubled size of the gluteal muscles, as well as over development of the deltoid

muscle. Deep grooves appear between the muscles. The twist may lack depth and fullness. The keeping quality of the meat is reduced because of the scanty covering of fat on the round which hastens drying out of the lean meat. The lack of marbling and coarseness of grain also decreases the demand for this kind of meat in the United States. In Holland, however, the butchers prefer double muscled carcasses, especially in veal. This character had appeared in the Alba sub-breed of the Piedmont cattle in Italy by 1886 (Paci, 81a) and is associated with greater efficiency of feed utilization than normal (Carbone, 11a). According to Wriedt (109) such calves are less vigorous than normal calves and more subject to rickets. They cause difficult birth. Although in Europe it appears most frequently in Friesians, it has been reported in Ayrshires in Norway, Shorthorns originating in Denmark, Charolais in France and the Piedmont cattle of Italy. In this country, Weber and Ibsen (104) have recorded its presence in Herefords, Aberdeen-Angus and Galloway. It is caused by one or more recessive genes.

*Hump.* In addition to the muscling in the thigh, another character dealing with muscular tissue is the hump at some location on the top-line characteristic of Indian cattle, *Bos indicus*. Nabours (78) described the large hump on the foreshoulders of the Indian cattle brought into Texas as weighing up 50 lb. and esteemed in India, England, and Russia as a delicacy for the table. Genes responsible for the hump were assumed by Nabours and others (40) to involve incomplete dominance. The observations that in Brown Swiss and Aberdeen-Angus crosses with certain Indian breeds the hump is more completely removed than in cases involving other European breeds, would lend support to the conclusion that several genes are incompletely dominant to the genes responsible for the shoulder hump.

Variations of the hump may be classified according to position, structure and function (19a). Bettini (8) described a typical muscular hump, thoracic in position, occurring in some of the Indian (Zebu) cattle. The inheritance of these differences are not yet known.

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## THE EFFECT OF HEAT TREATMENT ON THE PRO-OXIDANT ACTIVITY OF COPPER IN MILK

F. W. BERNHART AND ELIZABETH LINDEN

*Research and Development Department  
Nutritional Division, Wyeth Incorporated  
Mason, Michigan*

It has been known for many years that a trace of copper contamination in milk is a highly active pro-oxidative catalyst responsible for off-flavors (5). Milk as secreted and not subjected to metal contamination contains copper and other pro-oxidant metals which probably act in the same manner as added contaminants. Studies on milk indicate that practically all the copper is in unionized combination with protein and that the protein-copper complex is capable of exerting pro-oxidant effects (14). Usual reasons given for the effectiveness of high temperature treatment in improving the keeping qualities of milk and milk products are the production of reducing compounds, especially sulfhydryl groups and the inactivation of enzymes (10). Heat treatment of milk may increase flavor stability by reducing the pro-oxidant activity of copper through mechanisms independent of sulfhydryl groups or enzymes. Observations made by several investigators indicate that copper in milk is reduced in pro-oxidant properties as the result of heat treatment (6, 7, 9). During sterilization of evaporated milk in tinned containers, the pro-oxidant properties of 1-3 ppm of added copper are entirely eliminated (8). These reports, however, do not include investigations of the mechanisms through which copper is reduced in pro-oxidant properties by heat treatment of milk.

A study of the effect of heat treatment of milk on reducing the pro-oxidant effect of copper depends upon the availability of a method for determining the concentration of copper having this property. Estimation of the concentration of a catalyst is commonly done by determining the rates of a reaction mediated by the catalyst. The oxidation of reduced ascorbic acid by molecular oxygen, a reaction which is strongly catalyzed by copper and does not occur without a catalyst at pH values of less than 7.6 (2), is the reaction used in this work as a method of estimating the pro-oxidant copper concentration in milk. The rate of ascorbic acid destruction has been used as an index of pro-oxidant copper activity in testing the effects of complexing agents (11).

Firm conclusions in regard to the concentration of pro-oxidant copper activity are not possible in a complex medium such as milk, in which heat causes the formation of reducing substances and destroys enzymes which may influence ascorbic acid destruction. However, comparisons of ascorbic acid destruction between samples containing the same amount of added copper and subjected to the same heat treatment at temperatures of heating known to destroy enzymes (85° C. and above) allows tentative conclusions to be drawn.

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The experiments reported here were designed to investigate the reactions which occur during heat treatment of milk and cause a decrease in the concentration of copper having pro-oxidant activity.

#### METHODS AND PREPARATIONS

All glassware used for treatment and incubation of milk samples and preparation of reagents was rinsed with concentrated nitric acid and then rinsed with tap water, distilled water and water double-distilled from glass. The milk used was obtained daily from a 2000-gal. sample of cooled, unpasteurized skim milk previously processed and stored in stainless steel and tinned copper equipment by the methods commonly employed in milk plants. The average copper content of this milk supply, determined by the method of Bendix and Grabensetter (3), was 0.17 mg. per liter (average of duplicate determinations of three samples). Skim milk samples, free of metallic contamination, were obtained by milking directly into a wide mouth bottle and centrifuging in glass tubes.

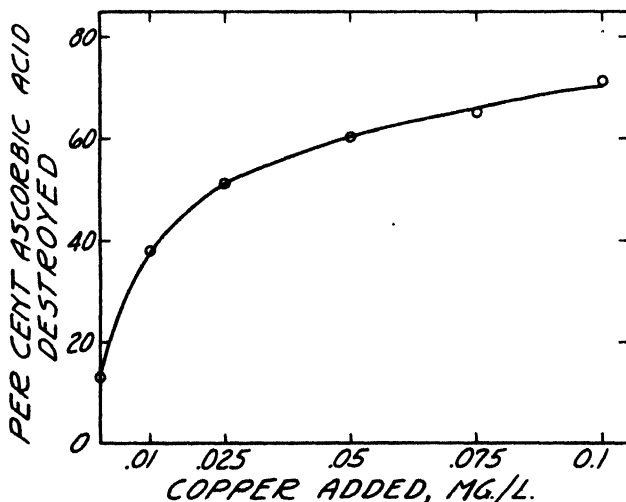


FIG. 1. The effect of copper on destruction of reduced ascorbic acid in aqueous solution during incubation for 2 hr. at 35° C.

Two hundred-ml. samples of milk, with and without added copper, were heated in 500-ml. Erlenmeyer flasks. After cooling to 55° F., copper was added to the required samples. Fifty-ml. aliquots in duplicate were measured accurately and placed in 500-ml. Erlenmeyer flasks. All samples then were placed in a 35° C. incubator until they had reached incubator temperature (about 2 hr.). To each sample was added 5 ml. of ascorbic acid solution, containing 5 mg. of ascorbic acid, and 0.5 ml. of toluene as a preservative. The samples then were shaken gently and allowed to incubate at 35° C. in the dark. Copper was supplied as copper sulfate. Reduced ascorbic acid was determined by the method outlined in A.O.A.C. Methods (1).



Casein was precipitated from skim milk by addition of HCl to pH 4.6. The washed precipitate was redissolved by addition of  $\text{NaHCO}_3$  solution. The solution, held in Visking casings under toluene, was dialyzed against 0.5 per cent  $\text{NaHCO}_3$  solution and running tap water for 48 hr. After filtering, the solution was dried by pervaporation and stored in a desiccator. Lactalbumin was prepared in a soluble form by dialysis of the filtrate obtained after precipitation of the casein. After dialysis against running tap water for 48 hr. using the same methods as described above for casein, the solution was concentrated by pervaporation to 0.1 volume, filtered and dried in the same manner as the casein. CP quality lactose was used.

#### RESULTS AND DISCUSSION

The data shown in fig. 1 demonstrates that, under the general conditions of these experiments, copper may be estimated through the pro-oxidant effect exerted upon the rate of oxidation of reduced ascorbic acid.

Milk samples heated at different temperatures, with copper added at various levels before heating and after heating, resulted in data shown in fig. 2. At each level of copper addition, the only variable was the time at which the copper was added. The data show that less ascorbic acid was destroyed during incubation in the samples to which copper was added before heating at  $121^\circ\text{C}$ . for 15 min. or  $104^\circ\text{C}$ . for 20 min. In samples heated at  $85^\circ\text{C}$ . for 20 min., the effect of the time of addition of copper was reversed.

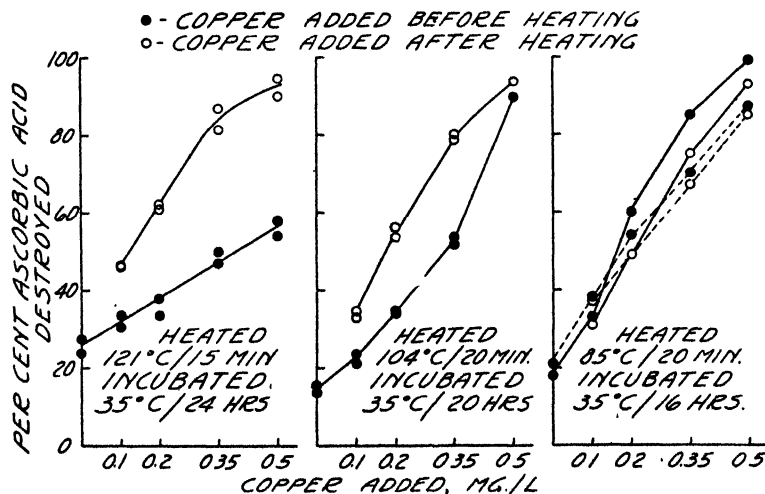


FIG. 2. Destruction of reduced ascorbic acid in milk heated to different temperatures as influenced by time and level of copper addition. Two experiments were performed on different days at each temperature.

Under the conditions of these experiments, the rate of destruction of reduced ascorbic acid was determined in preliminary experiments to approximate closely a first order reaction. To allow a direct comparison of the effects of copper added

to milk heated to different temperatures, the data shown in fig. 2 were recalculated to find the destruction of reduced ascorbic acid which would have occurred after 16 hr. incubation, assuming a first order reaction (fig. 3).

Further information on the difference in the effects of heat treatments can be shown by the following analysis of the data: Let  $A$  equal the amounts of ascorbic acid destroyed in samples to which the copper was added after heating, minus the control value (zero copper addition) and let  $B$  equal the ascorbic acid destroyed in samples to which the copper was added before heating, minus the control value. Then, if the ratios of  $A$  to  $B$  are plotted against the amount of copper added, approximate straight line relationships result. The equation of the

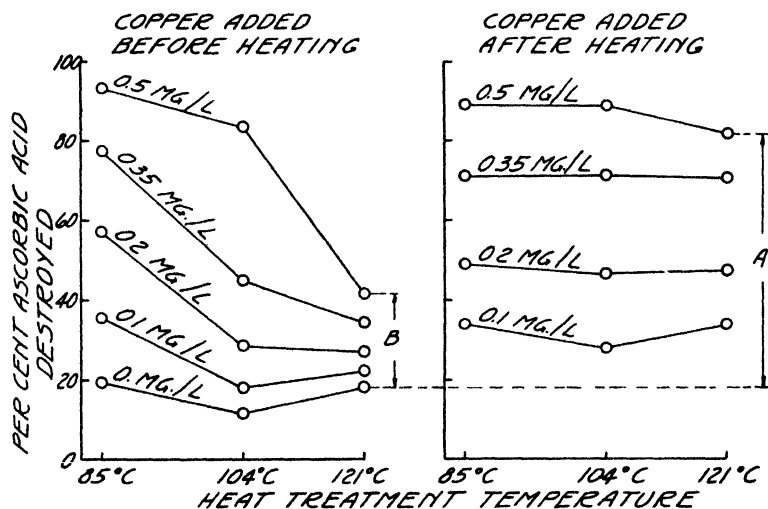


FIG. 3. The effects of heat treatment temperature, level of copper addition and time of copper addition on the destruction of reduced ascorbic acid in milk during 16-hr. incubation at 35° C. Calculated from data shown in fig. 2. The method of obtaining the values  $A$  and  $B$  (see fig. 4) are shown for one point.

lines is:  $A/B = Y + (Cu) \cdot K$ , where  $Y$  is the extrapolated value of the ratio at zero addition of copper,  $(Cu)$  = concentration of added copper in mg. per l. and  $K$  = slope of the line. These calculations are shown in fig. 4.

The values of the ratio  $A/B$  are highest at all levels of copper addition in the experiments in which the milk was heated at 121° C. for 15 min. Higher values of the ratio are related to the greater difference in the effect of the heat treatment on the destruction of ascorbic acid by copper added after heating as compared to the same amount of copper added before heating. The slopes of the lines obtained at the two higher heat treatments are negative, indicating that the same type of reactions occur at both temperatures. In contrast to the results obtained at the higher temperatures, a value for the ratio  $A/B$  of less than one is obtained after heating at 85° C. At this temperature copper added before heating causes more destruction of reduced ascorbic acid than copper added after heating. The slope of the line is slightly positive.

The most probable explanation of the results obtained at the higher temperatures of heating is that heating the milk with the copper causes the copper to be less available as a pro-oxidant in comparison with the pro-oxidant properties of copper added after heating. A less likely possibility is that the presence of added copper during heat treatment causes formation of greater amounts of substances stabilizing reduced ascorbic acid than are formed during heating without copper. As far as is known, no data is available to indicate the possibility of a pro-oxidant metal acting in this way. On the contrary, copper is a catalyst for the oxidation of SH groups (12). However, the data do not preclude the possibility that the less likely mechanism may be operative.

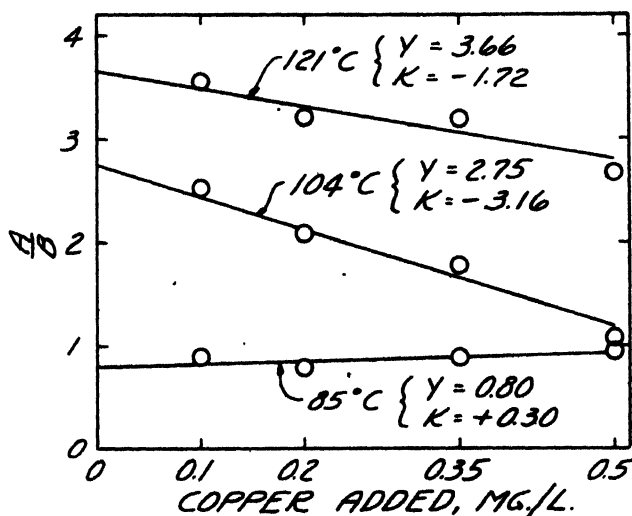


FIG. 4. The effect of temperature and level of copper addition to milk on the ratio of ascorbic acid destroyed due to copper added before heating, to ascorbic acid destroyed due to copper added after heating. Calculated from data shown in fig. 3.

The values obtained may be the result of two opposing types of reactions. At 85° C. a greater destruction of ascorbic acid occurs in samples to which copper is added before heating. This result indicates that copper, heated with milk at 85° C., is not reduced in pro-oxidant properties as compared to copper added after heating but rather causes reactions to occur which effect a decrease in the stability of reduced ascorbic acid and suggests that reactions which decrease the stability of reduced ascorbic acid may occur when copper is heated with milk at the higher temperatures. At 121 and 104° C., reactions responsible for the binding of pro-oxidant copper and hence stabilizing ascorbic acid may overbalance the effects of reactions resulting in a medium in which ascorbic acid is less stable. The difference in the slopes of the curves relating ascorbic acid destruction to temperatures of heating with increasing levels of copper added before heating (fig. 3) also indicate the occurrence of two opposing reactions.

Extrapolation of the lines to zero addition of copper yields values  $Y$  which are quantitative estimates of the relative effectiveness of heat treatments in decreasing the pro-oxidant activity of a minute quantity of copper added before heat treatment, as compared to a similar addition after heat treatment.

In order to determine which components of heated milk are responsible for the change in effect of copper on reduced ascorbic acid, the separated components of milk were tested. Solutions of 3 per cent casein, 0.5 per cent lactalbumin, and 5 per cent lactose were prepared. After adjustment to pH 6.8, copper was added to each solution at a level of 2 mg. per l. After thorough shaking and dividing into two equal portions, one set of solutions was autoclaved at 121° C. for 15 min. in sealed glass containers. The precipitates caused by heating were dispersed thoroughly by adding glass beads to the containers and shaking mechanically for 1.5 hr. Casein and lactalbumin solutions were prepared, as described above, and heated at 121° C. for 15 min. without copper. After cooling and mechanical shaking, copper then was added.

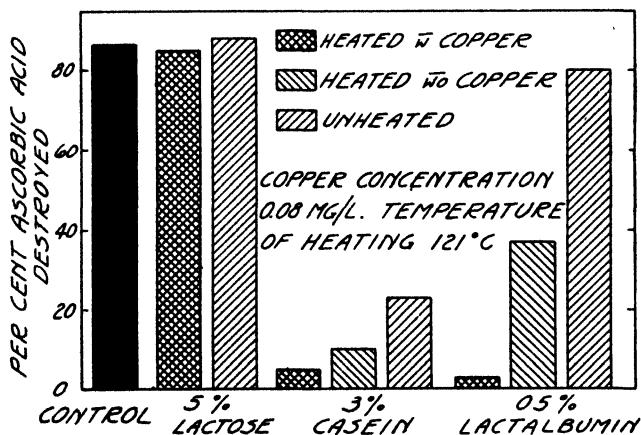


FIG. 5. Destruction of reduced ascorbic acid in 0.015  $M$  acetate buffer, pH 4.6, during incubation for 100 min. at 35° C. as influenced by copper as copper sulfate and copper added with separated milk components.

The effects on oxidation of reduced ascorbic acid in the solutions prepared above were tested by addition of 2 ml. of the samples containing 0.004 mg. copper to 48 ml. of 0.015  $M$  acetate buffer pH 4.6. To the control flask were added 2 ml. of copper sulfate solution containing the same amount of copper as the experimental samples. Five ml. of ascorbic acid solution (1 mg./ml.) and 0.5 ml. of toluene were added and the mixture incubated at 35° C. in the dark for 100 min. The data obtained are shown in fig. 5.

The data supports the interpretation that copper heated with lactose or added to heated lactose is unchanged in activity within the experimental error of the methods used. Copper added to unheated casein forms a copper-casein complex, in which form the copper is lowered considerably in pro-oxidant activity as meas-

ured by destruction of reduced ascorbic acid (23 per cent destroyed as compared to 86 per cent destroyed by the same amount of copper as  $\text{CuSO}_4$ ). Unheated lactalbumin reduces the pro-oxidant activity of copper considerably less than casein (80 per cent ascorbic acid destroyed as compared to 86 per cent destroyed in the control). Copper is further reduced in activity when added to previously heated casein solution (10 per cent reduced ascorbic acid destroyed) and lactalbumin solution (37 per cent reduced ascorbic acid destroyed). This further decrease of the pro-oxidant properties of copper added to heated protein, as compared to this property when copper is added to unheated protein, probably is due to the binding of the copper by sulfhydryl groups formed during the heat treatment (4), as sulfhydryl groups have been shown to bind copper (13). A further significant reduction in pro-oxidant activity occurs when copper and proteins are heated together. Copper heated with casein caused 5 per cent destruction of reduced ascorbic acid and copper heated with lactalbumin caused only 3 per cent destruction of ascorbic acid. The results obtained on heating copper with milk proteins confirm the explanation advanced for the loss of pro-oxidant properties of copper added to evaporated milk before sterilization (8). When the copper was heated with casein, only very slight coagulation occurred, indicating that copper-casein complexes of lowered pro-oxidant activity were formed. In the case of copper heated with lactalbumin, coagulation of the protein occurred. Probably at least part of the copper is mechanically removed from contact with the ascorbic acid solution by inclusion in the coagulum.

As a basis for further investigation, a theory is proposed to account for the data obtained. Heating milk at 85° C. or higher causes formation of substances, probably the most important of which are sulfhydryl groups, which reduce the pro-oxidant activity of copper and also may act as reduced ascorbic acid stabilizers. In addition to this mechanism for stabilizing reduced ascorbic acid, heat treatment of milk at 121 and 104° C. results in a further reduction in the pro-oxidant properties of copper through formation of complexes in which the copper is less capable of exerting pro-oxidant effects in comparison with the copper-protein and other combinations present in milk heated at 85° C. or lower temperatures. This inactivation probably is related to lactalbumin denaturation. Opposing these two reactions which stabilize ascorbic acid, the copper present in milk during heat treatment causes an increased rate of destruction of copper binding groups and/or groups stabilizing ascorbic acid which are formed during heating. As a result, reduced ascorbic acid is less stable. The over-all effect observed is the resultant of the effects of these reactions.

As a practical matter, the data (fig. 3) indicate that heat treatment at 104° C. is the optimum temperature of heat treatment for the milk supply used in these studies, as far as maximum stability of ascorbic acid in final products is concerned, though heat treatment at 85° C. is almost as good in this respect. Comparative tests done in this laboratory have shown that the stability of reduced ascorbic acid in milk samples previously heated at 85° C. is greater than in milk samples previously heated to lower temperatures.

To investigate the effect of metallic contamination resulting from contact of

the milk with metallic equipment, the heat treatment at 121° C. for 15 min. was applied to milk samples handled exclusively in glass. As shown in fig. 6, considerably less ascorbic acid was destroyed in all samples tested, as compared to milk subjected to metallic contaminants. The reduced ascorbic acid destroyed is related directly to the copper added before heating, as was found previously at 121° C. with milk handled in metallic containers.

Extrapolation of the straight lines obtained upon plotting reduced ascorbic acid destruction against added copper to zero destruction of ascorbic acid also is shown in fig. 6. This extrapolation is justified if the assumption is made that the

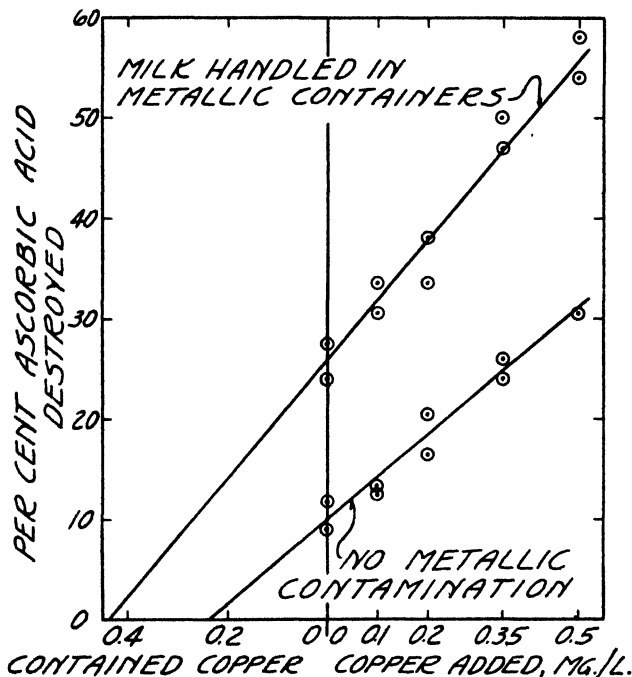


FIG. 6. The effect of adding copper to milk handled in glass and in metallic equipment on destruction of reduced ascorbic acid during 24-hr. incubation at 35° C. The copper contents of the milk samples are estimated by extrapolation.

pro-oxidant metal content of the milk acts in a similar manner to added copper. It is of interest that extrapolation furnishes a value of about two times as much copper in milk previously in contact with metals (0.43 mg./l.) as compared to the milk samples held in glass (0.24 mg./l.). The copper content of the milk previously in contact with metal obtained by this calculation (0.43 mg./l.) is over twice the value obtained by chemical analysis (0.17 mg./l.). Iron, manganese and other metals acting as pro-oxidants in the presence of copper may be the reason for the higher value for copper obtained by the extrapolation. It is suggested that a test based on these observations might be developed for estimation of total pro-oxidant metal activity of milk samples.

## SUMMARY

Copper added to milk before heat treatment at 121° C. for 15 min. or 104° C. for 20 min. causes lower rate of disappearance of ascorbic acid on subsequent incubation than is caused by copper added after heating. The reverse effect occurs in samples heated at 85° C. for 20 min.

Approximately straight line relationships are obtained when the amounts of reduced ascorbic acid destroyed above the control value in milk samples to which the copper is added after heating are divided by the amounts destroyed above the control value in similar samples to which the copper is added before heating, and these values plotted against the amount of copper added. Extrapolation of the lines to zero addition of copper affords a quantitative estimate of the effect of slight copper contamination occurring before heat treatment, as compared to the same level of copper contamination occurring after heat treatment.

Casein-copper and lactalbumin-copper mixtures heated at 121° C. for 15 min., when added to ascorbic acid solutions, cause a lower rate of loss of reduced ascorbic acid than do the unheated mixtures. If copper is added to previously heated protein solutions, an effect on reduced ascorbic acid oxidation intermediate between heated and unheated samples occurs.

These data furnish evidence that high-temperature heat treatment of milk reduces the pro-oxidant effects of milk copper and allows an interpretation of the mechanisms to be advanced.

Extrapolation to zero of the straight lines obtained on plotting the percent ascorbic acid destroyed against the copper added to milk samples subsequently heated at 121° C. for 15 min. yields values for copper approximately twice as great as were obtained by chemical analysis.

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# A PICRIC ACID METHOD FOR THE SIMULTANEOUS DETERMINATION OF LACTOSE AND SUCROSE IN DAIRY PRODUCTS<sup>1</sup>

N. A. PERRY<sup>2</sup> AND F. J. DOAN

*The Pennsylvania Agricultural Experiment Station  
State College*

For the determination of lactose in milk, "Official Methods" of the A.O.A.C. (1) recognizes a polarimetric method (Double Dilution) and a copper reduction method (Munson-Walker). With sweetened condensed milk the Munson-Walker method is specified for lactose, while a polarimetric method, requiring readings before and after hydrolysis, is the only procedure mentioned for sucrose. No sugar methods are included for such products as cream, ice cream, and dry milk.

White (18) has developed a procedure based on the Munson-Walker technique which can be used for lactose and sucrose when present together. This method corrects for certain irregularities in the unmodified Munson-Walker method and has been found more accurate and convenient than "Official Methods" for sweetened condensed milk (13, 10).

At best, the recognized methods for lactose and sucrose in dairy products are tedious, lengthy, or require specialized equipment and for this reason sucrose determinations often are avoided.

Colorimetric methods for certain carbohydrates, based on the reduction of picric acid, have been developed and used for many years, particularly for glucose in blood and urine (2, 5, 6, 9, 13, 15), but later for carbohydrates in plant materials (16, 17, 19) and for lactose in milk (3, 10, 12) and other dairy products (3). These methods have proven simpler than the official ones and on the whole quite satisfactory. Most of them, however, were evolved using visual colorimeters (less sensitive and less accurate than present photoelectric instruments) and in many cases the color development was not strictly proportional to the concentration of sugars, thereby necessitating correction factors, standard curves or standard colors close in color density to the unknowns.

In the belief that a satisfactory colorimetric technique, based on the reduction of picric acid, for the simultaneous determination of lactose and sucrose in dairy products would be a very useful tool in dairy products control and in dairy products research, the study reported here was undertaken.

## EQUIPMENT AND METHODS

A Klett-Summerson photoelectric colorimeter was used for all color comparisons, employing a no. 52 filter (520 m $\mu$ ) which was found to be slightly more sensitive than the no. 54 recommended by the maker of the instrument for picric

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<sup>2</sup> Present address: Mid-west Dairy Products Corp., Cape Girardeau, Missouri.

creatinine determinations. The scale of this instrument is graduated logarithmically and the readings are proportional to color density and hence to concentration of the substance responsible for the color.

A water bath equipped with a peripheral perforated steam pipe and an overflow made it possible quickly to obtain boiling water at a constant level for heating flasks and tubes. In this bath (14" × 8" × 8") the introduction of a series of tubes or flasks did not lower significantly the temperature of the water.

In the early studies, flasks (100 ml.) and various tubes were used, as called for by the method or modification under investigation, but later the "sugar tubes" suggested by Meyers and Bailey (14) were adopted because of their greater convenience. These are straight-sided, 200 × 15 mm. tubes graduated at 3, 4, 10, 15 and 20 ml.

Standard lactose solutions were prepared from dried C.P. lactose monohydrate on which triplicate polarimetric determinations indicated a purity of 99.88 per cent.

Standard sucrose solutions were made from a supply furnished by the National Bureau of Standards (lot 4602, standard sample 17) analyzing less than 0.003 per cent moisture, 0.003 per cent ash and less than 0.002 per cent invert sugar. A portion of this was subjected to twice-repeated crystallization with absolute alcohol from concentrated water solution, followed by vacuum drying over  $P_2O_5$ . Samples of the original and "purified" sugar tested by the procedure of Willaman and Davison (19) gave identical readings.

The picric acid employed (Baker's, C. P., Analyzed, Crystalline, Special for blood test) was found satisfactory by the Folin and Doisy test (7).

The first part of the work consisted of a reexamination of the picric acid methods of Myers and Bailey (14), Benedict and Osterberg (2), Bierman and Doan (3) and Willaman and Davison (19) in an effort to select a procedure most satisfactory as a basis for determining lactose and sucrose in dairy products. The methods were modified in various ways (largely by altering the amount and concentration of reagents and by varying the heating periods) in an effort to obtain color development in conformity to Beer's law which none of them exhibited initially.

The second portion of the work consisted of comparative determinations of lactose and of lactose and sucrose in milk, lactose adjusted milk, simulated sweetened condensed milk and ice cream mix. The methods compared were the official Munson-Walker, the official polarimetric and the picric acid method developed in this study, where lactose alone was being determined. Where lactose and sucrose were being determined, White's (18) copper reduction method only was used as the standard for comparison with the picric acid method. Ten replicate determinations were made by each of the above methods on each of the 19 samples analyzed and the data were examined statistically.

## RESULTS

*Development of Method.* The detailed studies made in this portion of the work are not reported nor are data shown, but a number of important observa-

tions should be mentioned, inasmuch as they affect the determination by the picric acid method and since they influenced the selection of a procedure. The color development, on which the method is based, is substantially complete when lactose or invert sugar is heated in the presence of picric acid and sodium carbonate for 20 min. However, it is not absolutely complete and therefore the heating period must be controlled. Blanks or standards preferably should be heated along with unknowns. Color, after development, is relatively stable (constant for 25 min.) but it does fade slowly. Consequently, samples should be limited to a number which can be read in the colorimeter in a 20-min. period. In this study 20 samples were found to be the maximum.

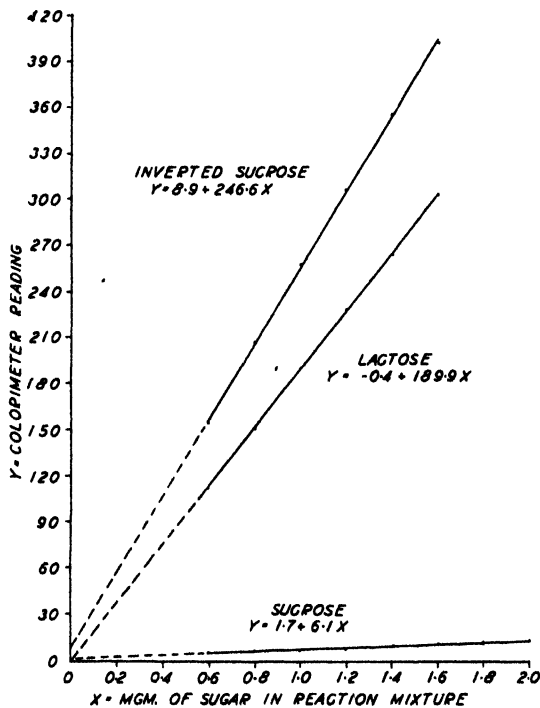


FIG. 1. Regression curves for lactose, sucrose and inverted sucrose.

*Lactose.* While none of the original methods produced color density in proportion to the concentration of sugar for the full length of the curve, a few of the modifications did. In general, increased heating periods increased the slope of the curve (concentration of sugar =  $X$  and color density =  $Y$ ) and also shifted the point of origin in a positive direction. A modification of the method of Willaman and Davison finally was selected as the most suitable, of those giving results conforming to Beer's law, for use in analyzing milk. A calibration curve and regression equation for lactose (fig. 1) was established from the analysis of

seven series of samples each containing six concentrations of pure lactose varying from 0.6 to 1.6 mg. in the reaction mixture. This equation is as follows:  $Y = -0.4 + 189.9 X$ , where  $Y$  is the colorimeter reading,  $-0.4$  the origin of the curve, and  $189.9$ , the increase of reading for each milligram of lactose (slope).

*Lactose and sucrose.* The literature (16, 19) states that sucrose is easily and quickly inverted by heating in picric acid and, further, that for reasonable periods at room temperature picric acid does not invert sucrose even in saturated solution (3, 16, 17). These facts were verified. Sucrose was found to be completely inverted in sugar tubes heated in the boiling water bath for 4 min. and saturated picric acid caused no measurable hydrolysis of sucrose at room temperatures over a period of 30 min. These observations are important because in the determination of lactose and sucrose in a medium where both are present colorimeter readings are made twice, once without inversion of sucrose and once after sucrose inversion.

Under these conditions it is necessary to know whether uninverted sucrose exhibits any reducing properties when heated in alkaline picrate solution. The literature suggests that it does not but repeated, careful trials in this work demonstrated that it does have a slight but significant effect. For accurate work, therefore, lactose determinations on products containing sucrose must be corrected. Since the reducing power of uninverted sucrose was found to increase with the alkalinity and to be proportional to the amount of sucrose present, this phenomenon apparently is the result of a slight degradation of the sucrose brought about by heating in alkaline solution, giving rise to fragments with reducing power. White (18), in his study of the Munson-Walker method for sweetened condensed milk, found the same error in that method and his modification corrects for it.

Previous workers found that the heat treatment in picric acid, used for inverting sucrose, does not affect the reducing power of lactose and other reducing sugars if also present. This fact was confirmed.

A considerable amount of time and effort was expended in attempting to find conditions for the reduction of picrate by inverted sucrose which would result in a color regression curve originating at zero and still not complicate the procedure or change it too radically from the simple procedure found satisfactory with lactose. This effort was not successful and it was finally decided to use the lactose procedure and adjust the concentration of sucrose in the sample to the portion of the color curve away from the lower readings.

A calibration curve and regression equation was established from data obtained in a similar manner as with lactose alone, but for both uninverted sucrose (correction on lactose) and inverted sucrose. These curves also are plotted in figure 1. For uninverted sucrose,  $Y = 1.7 + 6.1 X$ . For inverted sucrose,  $Y = 8.9 + 246.6 X$ .

#### METHOD

*Reagents.* Saturated picric acid is prepared by heating 16 g. of the wet, C.P., reagent (special for blood test) per liter of distilled water until dissolved, then

cooling it slowly at room temperature for several days until the excess solute has crystallized in the form of large, blade-like crystals. Quick cooling and agitation are conducive to fine, powdery crystals difficult to keep out of the supernatant liquid which is the reagent.

Twenty-five g. of C.P., anhydrous  $\text{Na}_2\text{CO}_3$  are dissolved and made up to 100 ml. with distilled water. This solution should not be allowed to cool much under  $65^\circ\text{F}$ . or some of the solute will crystallize.

*Where lactose is the only sugar.* Approximately 1 g. of milk, cream, skim-milk or a proportionate amount of other product, is weighed accurately into a 100-ml. volumetric flask and diluted to volume with saturated picric acid. The flask contents are shaken and filtered through a fluted filter. Two ml. of the filtrate are transferred to a Myers and Bailey sugar tube containing 1 ml. of the  $\text{Na}_2\text{CO}_3$  reagent, stoppered lightly, shaken and placed in a bath of boiling water for 20 min. The tube then is cooled to room temperature (approx.  $20^\circ\text{C}$ .), diluted to 20 ml. with distilled water and mixed by inverting. A portion is transferred to a colorimeter tube and a reading obtained within 20 min. of removal from bath. A blank consisting of 2 ml. of picric acid and 1 ml. of  $\text{Na}_2\text{CO}_3$  is heated, cooled and diluted along with the unknown for adjustment of the zero point of the colorimeter scale.

The calculation is simple where lactose is the only sugar present. The value of  $X$  is first determined from the regression equation for lactose,  $X = \frac{Y + 0.4}{189.9}$ , where  $X$  = mg. of lactose in 2 ml. of filtrate and  $Y$  = photoelectric colorimeter reading. From this, the amount of lactose in the unknown is calculated as follows: % lactose = 
$$\frac{X \cdot 100 \cdot 100}{1000 \cdot 2 \cdot \text{wt. of sample}} = \frac{5X}{\text{wt. of sample}}$$

*Where lactose and sucrose are both present.* Approximately 5 g. of sweetened condensed milk, 13 g. of ice cream or an amount of other product giving a comparable amount of sucrose are weighed into a 100-ml. volumetric flask and diluted to volume with distilled water. The contents are well mixed and a 5-ml. portion transferred to another 100-ml. flask and made to volume with saturated picric acid, mixed and filtered. Then 2 ml. of the filtrate are introduced into a "sugar tube" containing 1 ml. of  $\text{Na}_2\text{CO}_3$  reagent, mixed and lightly stoppered.

Another 1-ml. portion of the same filtrate is transferred to a sugar tube containing 1 ml. of saturated picric acid, mixed well and placed in the boiling water bath for 5 min., after which it is cooled to room temperature. This second tube now contains inverted sucrose. One ml. of  $\text{Na}_2\text{CO}_3$  is added to it and both tubes are placed in the boiling water bath for 20 min., along with a blank for each of them consisting of 2 ml. of saturated picric acid plus 1 ml. of carbonate for the first and 1 ml. of each reagent for the second. All the tubes then are cooled to room temperature, diluted to 20 ml. with distilled water and transferred to colorimeter tubes for reading. The instrument scale is set at zero using the proper blank before the respective readings are made.

The calculations required when both sugars are present are considerably more involved, even when results for only one are desired, inasmuch as a correction

must be made for the effect of uninverted sucrose on the lactose reading. The following symbols are used in explaining these calculations:  $X_1$  = mg. lactose in 2 ml. of filtrate,  $X_2$  = mg. sucrose in 1 ml. of filtrate,  $Y_1$  = colorimeter reading before inversion of sucrose,  $Y_2$  = colorimeter reading after inversion of sucrose and  $Y_3$  = portion of  $Y_1$  reading due to action of uninverted sucrose. Using the regression equation for inverted sucrose,  $Y = 8.9 + 246.6 X$  or  $X = \frac{Y - 8.9}{246.6}$ , an estimated value for  $X_2$ , sufficiently accurate for calculating the value  $Y_3$ , is obtained, thus:

$$X_2 \text{ (est.)} = \frac{Y_2 - \frac{Y_1}{2} - 8.9}{246.6}$$

The amount of sucrose present in the reaction mixture when the color due to lactose is being developed is twice this amount or  $2X_2$  (est.). The regression equation for uninverted sucrose,  $Y = 1.7 + 6.1 X$ , then becomes  $Y_3 = 1.7 + 6.1 [2X_2 \text{ (est.)}]$ . Substituting the value of  $X_2$  (est.), one obtains  $Y_3 = 1.7 + 6.1$

$$\left[ 2 \left( \frac{Y_2 - \frac{Y_1}{2} - 8.9}{246.6} \right) \right], \text{ which may be simplified to } Y_3 = \frac{Y_2 - \frac{Y_1}{2} + 25.4}{20.2}$$

The regression equation for lactose,  $Y = -0.4 + 189.9 X$  or  $X = \frac{Y + 0.4}{189.9}$ , now is employed and  $X_1 = \frac{Y_1 - Y_3 + 0.4}{189.9}$ . Consequently, the lactose in the sample can be calculated as follows:

$$\% \text{ lactose} = \frac{X_1 \cdot 100 \cdot 100 \cdot 100}{1000 \cdot 2 \cdot 5 \cdot \text{wt. of sample}} = \frac{100 X_1}{\text{wt. of sample}}$$

The regression equation for inverted sucrose again is used for calculating accurately the value of  $X_2$ , and hence the amount of sucrose in the sample:

$$X_2 = \frac{Y_2 - \frac{Y_1 - Y_3}{2} - 8.9}{246.6}; \text{ and } \% \text{ sucrose} = \frac{X_2 \cdot 100 \cdot 100 \cdot 100}{1000 \cdot 1 \cdot 5 \cdot \text{wt. of sample}} = \frac{200 X_2}{\text{wt. of sample}}$$

The regression curves and methods of calculation used in the described method should be applicable to any colorimeter using a scale similar to the Klett-Summerson (logarithmic). For other colorimeters, calibration curves obtained with pure sugar solutions can be employed. Such curves (fig. 1) also may be used, if desired, with logarithmic scales.

#### ACCURACY OF METHOD

**Lactose.** The picric acid method, as described, was employed in analyzing nine samples of milk and lactose-adjusted milk for lactose alone and the results compared with those obtained by means of the official Munson-Walker method and the official polarimetric method. Ten replicate determinations were made by each method on each sample. The results are summarized in table 1. These show excellent agreement of the average results by all three methods. The picric

acid method gives results intermediate between the two official methods but the results are slightly more variable than for either of them.

Analysis of variance of the original data indicates that differences between replicates are not significant. Variations among methods, using the interaction between methods and replications, are significant. The difference between the average per cent of lactose by the picric acid and the polarimetric methods is not

TABLE 1

*Accuracy and variability of the picric acid method as compared with the Munson-Walker and polarimetric methods on milk where lactose only is being determined*

Method	Lactose <sup>a</sup> (%)	Standard deviation	Coefficient of variation (%)
Picric acid	5.929	0.055	0.93
Munson-Walker	5.904	0.044	0.74
Polarimetric	5.932	0.044	0.74

<sup>a</sup> Av. of 90 determinations by each method.

significant; that between the picric acid and the Munson-Walker methods is significant; that between the polarimetric and the Munson-Walker methods is highly significant. For ordinary analytical purposes these differences are believed to be inconsequential.

Four of the samples analyzed in this comparison were obtained by adding lactose to two normal samples of milk. The recoveries of these added quantities of this sugar by all three methods used are indicated in table 2. These are exceptionally close to the expected values.

TABLE 2

*Recovery of added lactose in four samples of milk by the three methods of analysis*

Method	Sample					
	Normal	Added lactose		Normal	Added lactose	
Picric acid method						
% lactose found	4.83	6.23	7.62	4.74	6.04	7.33
% lactose expected		6.24	7.60		6.05	7.32
% of expected		99.8	100.3		99.8	100.1
Munson-Walker method						
% lactose found	4.78	6.17	7.54	4.68	6.03	7.29
% lactose expected		6.19	7.55		5.99	7.26
% recovery		99.7	99.9		100.7	100.4
Polarimetric method						
% lactose found	4.81	6.21	7.62	4.78	6.07	7.37
% lactose expected		6.22	7.58		6.09	7.36
% of expected		99.8	100.5		99.7	100.1

*Lactose and sucrose.* White's copper reduction method for lactose and sucrose was the method used for comparative purposes with the picric acid method where both sugars were determined. Ten replicate determinations were made on each of ten different samples of ice cream mix, simulated sweetened condensed milk and sweetened condensed milk. The ratios of sucrose to lactose in the ice

cream mix samples and the simulated sweetened condensed milk were of such a range as to cover all possibilities likely to be encountered in commercial products. The results obtained for lactose in these products are summarized in table 3.

The data indicate excellent agreement between the two methods but, as in the previous comparison, the pieric acid method exhibits results which are slightly more variable than the method used for comparison. Analysis of variance reveals no significant difference between the mean values obtained by the two methods.

TABLE 3

*Accuracy and variability of the pieric acid method as compared with White's modification of the Munson-Walker method for lactose in dairy products containing sucrose*

Method	Lactose <sup>a</sup>	Standard deviation	Coefficient of variation
	(%)		(%)
Pieric acid	5.409	0.062	1.15
White's	5.396	0.053	0.98

<sup>a</sup> Average of 100 determinations by each method.

The results obtained for sucrose on these same samples are presented in summarized form in table 4. Analysis of variance of these data does indicate a significant difference between the mean values obtained by the two methods. Most analysts probably would consider even the widest variations obtained in the study (0.33 per cent for a sample of commercial, sweetened condensed milk) a reasonably small error for sucrose determination. As the data show, the pieric acid method gives somewhat more variable results than the method of White.

TABLE 4

*Accuracy and variability of the pieric acid method as compared with White's modification of the Munson-Walker method for sucrose in dairy products*

Method	Sucrose <sup>a</sup>	Standard deviation	Coefficient of variation
	(%)		(%)
Pieric acid	16.609	0.114	0.69
White's	16.694	0.096	0.58

<sup>a</sup> Average of 100 determinations by each method.

Six of the ten samples analyzed for both lactose and sucrose were samples of ice cream mix and simulated sweetened condensed milk, where the lactose content of the milk and condensed milk used in their preparation was known and the amount of sucrose added was weighed carefully. Consequently it was possible to compare the values obtained by the pieric acid method and White's method with expected values for sucrose. These comparisons are presented in table 5. The results show excellent agreement except for one value by White's method.



TABLE 5

*Recovery of added sucrose in six samples of ice cream mix and simulated sweetened condensed milk by two methods of analysis*

Ratio of sucrose to lactose	Sucrose expected	Picric acid method		White's method	
		Sucrose found	Per cent of expected	Sucrose found	Per cent of expected
	(%)	(%)		(%)	
2.40-1.0	10.43	10.49	100.6	10.57	101.3
2.65-1.0	11.39	11.43	100.4	11.35	99.6
2.90-1.0	12.33	12.31	99.8	12.28	99.6
3.30-1.0	12.93	12.88	99.6	12.99	100.5
3.65-1.0	14.11	14.08	99.8	14.15	100.3
4.00-1.0	15.25	15.19	99.6	15.27	100.1

## CONCLUSIONS

As a result of detailed studies of the procedures recommended in the literature for the colorimetric determination of sugars by the reduction of picric acid or sodium picramate, a method employing this technique but utilizing a photoelectric colorimeter was evolved for the simultaneous determination of lactose and sucrose in dairy products. The method also is applicable to the determination of either sugar alone.

The picric acid method is empirical and time factors must be controlled carefully. The technique is relatively simple as only two easily prepared reagents are required and, compared with commonly used methods, it is less tedious and less time consuming. The calculations, where both sugars are present, are somewhat involved but not difficult to apply.

Comparative studies indicate that the picric acid method probably is as accurate as the Munson-Walker, the polarimetric or White's modification of the Munson-Walker, although analysis shows that the variation of replicates is slightly wider.

It is felt that the method offers distinct advantages over currently used procedures for sugar determinations in a number of dairy products. It also appears simpler, especially insofar as reagents are concerned, than the modified ferri-cyanide procedure of Hites and Ackerson (8) and at least as convenient as the saccharimetric-refractometric method of Browne (4).

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# THE EFFECTS OF MILD INBREEDING ON A HERD OF HOLSTEIN-FRIESIAN CATTLE<sup>1</sup>

R. H. NELSON<sup>2</sup> AND J. L. LUSH

*Iowa Agricultural Experiment Station, Ames*

Inbreeding, in some instances, has been an effective system of breeding for livestock improvement but never has come into very common use in purebred herds and flocks, presumably because the incidence of abnormalities and other undesired traits is higher among inbred individuals. In recent years and at the present time, inbreeding projects have been and are being initiated by many experiment stations to obtain a more conclusive answer on how to derive greater benefit from this system of mating. With few exceptions, these experiments with large animals, as well as those with laboratory animals, have resulted in reduced vigor as exhibited by slower growth, smaller size, greater mortality and lower production and fertility. The present paper reports the effects of mild inbreeding on several characteristics in a herd of Holstein-Friesian cattle.

## DATA AND METHOD USED

A project with registered Holstein cattle was inaugurated by the Iowa Agricultural Experiment Station in 1930 to determine the consequences of mild inbreeding accompanied by selection for high production. This study is an analysis of the records accumulated in this project between 1930 and 1942.

The herd was closed to outside blood in 1930 except for three proven sires used briefly in 1932 to 1935. Usually about 35 to 50 females of breeding age were maintained in the herd, the number being larger in the later years. The breeding system followed was to use sons of the best producing cows and to keep them in service until sufficient cows (30 or more) were bred to each one to be fairly certain he would have at least eight tested daughters. This resulted in most of the bulls being used for slightly over 1 yr. each. This breeding system would be expected to raise the inbreeding something like 3 per cent per generation or a little under 1 per cent per year (14). An analysis of the pedigrees indicates this is approximately the amount of inbreeding that did occur. Inbreeding was measured by Wright's (13) formula and was computed from pedigrees traced in all lines to animals born in 1910 or earlier. Inbreeding coefficients ranged from 0 to 28 per cent, with the majority of the animals falling in the lower third of the range.

In addition to birth weights of both bull and heifer calves, the following measurements were taken on females at 6 mo. and 1, 2, 3, 4, 5 and 7 yr. of age:

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<sup>2</sup> Now Professor of Animal Husbandry, Michigan State College, East Lansing.

wither height, chest depth, body length, heart girth, paunch girth and weight. Weight was recorded in pounds and all other measurements in centimeters. Production records were kept on all cows. These records were converted to a 305-day mature equivalent basis. During much of the period covered by this study, the herd was on 3 times milking; hence the records made on 2 times or 4 times milking were converted to the 3 times basis. All animals over 6 mo. of age were classified for type each year, using judges who did not know this herd but were experienced. In most cases these judges were official classifiers for the breed association, although these classifications were not made official. These type ratings were used to study the effect of inbreeding on type. Correlation coefficients and the regressions of the various characters on inbreeding were used to measure the effect of inbreeding. Coefficients referred to as being statistically significant are those expected to occur by chance less than 5 per cent of the time.

The animals included in the study were sired by 26 different bulls. An analysis of variance of each of the measurements indicated that in most cases the

TABLE 1  
*Effect of inbreeding on weight at various ages*

Age	No. animals	Av. inbreeding	Av. weight	No. sires	Intra-sire	
					Correlation	Regression
		(%)	(lb.)			
Birth (males)	179	6	92	17	- 0.03	- 0.09
Birth (females)	191	5	86	25	- 0.08	- 0.16
6 mo.	176	5	354	19	- 0.08	- 0.72
1 yr.	168	5	638	23	- 0.22 <sup>b</sup>	- 2.74 <sup>b</sup>
2 yr.	153	5	1085	24	- 0.24 <sup>b</sup>	- 4.75 <sup>b</sup>
3 yr.	127	4	1192	26	- 0.14	- 4.00
4 yr.	90	3	1276	21	- 0.26 <sup>a</sup>	- 2.89 <sup>a</sup>
5 yr.	65	3	1329	20	+ 0.19	+ 5.46

<sup>a</sup>  $0.01 < P < 0.05$ .

<sup>b</sup>  $P < 0.01$ .

differences between sires caused a significant portion of the variation in measurements. To eliminate these sire differences and to keep all coefficients comparable, all of the correlations and regressions were calculated on an intrasire basis. Since all of a sire's progeny tended to be born within a period of slightly over 1 yr. with the breeding system followed in this herd, calculation on an intra-sire basis tended to eliminate any time trends resulting from changes in management.

#### RESULTS AND DISCUSSION

No definite lethals were found. The very few distinct abnormalities observed were slightly (but insignificantly) more abundant among the non-inbred calves, but the inbreeding was scarcely extreme enough to reveal rare recessive single-gene defects on a detectable scale anyway.

**Birth weight.** The birth weights were analyzed to determine the effect of factors other than inbreeding. Age of dam had a significant effect on weight at birth. Calves from 2-yr.-old heifers averaged 8 lb. less at birth than calves from

older cows. This amount was added to the birth weights of the calves from 2-yr.-olds to make those suitable for inclusion in the analysis of the effect of inbreeding.

The average inbreeding, birth weights and the intra-sire correlations and regressions of birth weight on inbreeding are included in table 1 for the 191 heifer and 179 bull calves. The weighted average of the bull and heifer regressions shows a decrease of about one-eighth of a pound in birth weight for each increase of 1 per cent in inbreeding. This value is just below the level of statistical significance. On the basis of this regression, a first generation of parent-offspring or full-sib matings would be expected to lower average birth weight by about 3 lb.

The effect of the inbreeding of the dam on the birth weight of the calf also was studied. Using the same birth weights as above, the regression of birth weight on inbreeding of the dam was  $-0.05$  for heifer calves and  $-0.14$  for bull calves. The weighted mean of the two gives a decrease of approximately one-eleventh of a pound for each increase of 1 per cent in inbreeding. This also is below the level of significance but is quite similar to the coefficient obtained for the effect of the inbreeding of the individual itself on its birth weight.

Though no definite conclusions can be drawn from these results, they are similar to those found by Woodward and Graves (12), Dickerson (5) and Tyler *et al.* (10), all of whom reported a decline in birth weight with increased inbreeding. Bartlett *et al.* (2, 3, 4, 9) reported that inbreeding had no apparent effect on birth weight in the Holstein herd they studied.

*Weights at 6 months and over.* The effect of inbreeding on weight at the various ages is shown by the regression coefficients of table 1. These show that, in general, increased inbreeding resulted in lighter weights at all ages except 5 yr. The regression coefficient increased up to 2 yr. Then, although the mean weight continued to increase, the regression coefficients decreased with advancing age up to 5 yr. This indicates that inbreeding not only resulted in lighter calves at birth, but in slower gains during the first 2 yr. of life. After 2 yr., the inbreds apparently gained faster than the non-inbreds and by 5 yr. of age approached or exceeded the non-inbreds in weight. Inbreeding evidently slowed the gains at younger ages but did not influence mature weight. The non-inbred individuals merely approached their mature weights at a younger age. The regression coefficient at 5 yr. indicates that mature body weight increased as inbreeding increased but the volume of data was not sufficient to make this trend significant.

As compared with earlier Holsteins from the same herd, (table IV in Espe *et al.*, 6) the ones in the present study were about 4 per cent lighter at 1 yr. and about 3 per cent heavier at 3 yr. and at 4 yr. At other ages the averages differed by only about 1 per cent.

Several have reported on the effect of inbreeding on growth rate. Bartlett *et al.* (2, 3, 4, 9) observed that growth rates of inbreds with a coefficient of inbreeding of less than 20 per cent were not significantly different from outbreds, although the outbreds showed a slight advantage at 2 yr. and the inbreds at 5 yr. They also found that animals with more than 20 per cent inbreeding were

smaller at maturity than outbreds. Dickerson (5) stated that the size difference in favor of outbreds appeared to become proportionately smaller with growth up to 6 mo. of age. In an inbreeding project with Guernseys and Holsteins, Woodward and Graves (12) observed that inbreeding tended to reduce mature weight. Baker *et al.* (1) analyzed the growth records on 63 daughters of one Holstein bull and found that weight decreased with increased inbreeding at all ages.

TABLE 2  
*Average measurements at various ages*

Age	No. animals	Av. inbreeding	Wither height	Body length	Chest depth	Heart girth	Paunch girth
(yr.)		(%)	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)
0.5	176	5	100	107	47	121	149
1	168	5	116	131	58	151	181
2	153	5	130	152	69	183	223
3	127	4	134	159	72	188	231
4	90	3	135	163	74	192	237
5	65	3	136	165	75	195	240

*Body measurements.* The results for the five body measurements were all quite similar and therefore will be presented as a group. The number of animals available for study, their average inbreeding and the average of each of the five measurements at the various ages are given in table 2. These figures show that average size continued to increase to at least 5 yr. of age. The averages for wither height and for chest depth are almost the same as those for earlier Holsteins in the same herd (table V in Espe *et al.*, 6). Wither height is a

TABLE 3  
*Intra-sire correlation coefficients between body measurements and inbreeding at various ages*

Age	No. sire groups	Wither height	Body length	Chest depth	Heart girth	Paunch girth
(yr.)						
0.5	19	-0.07	-0.14	-0.17 <sup>a</sup>	-0.15	-0.12
1	23	-0.16	-0.19 <sup>a</sup>	-0.23 <sup>a</sup>	-0.20 <sup>a</sup>	-0.17 <sup>a</sup>
2	24	-0.05	-0.18 <sup>a</sup>	-0.21 <sup>a</sup>	-0.22 <sup>a</sup>	-0.22 <sup>a</sup>
3	26	+0.14	-0.08	+0.08	0.00	-0.13
4	21	+0.31 <sup>b</sup>	-0.08	+0.19	+0.05	-0.10
5	20	+0.38 <sup>b</sup>	+0.06	+0.32 <sup>a</sup>	+0.19	+0.26

<sup>a</sup> 0.01 < P < 0.05.

<sup>b</sup> P < 0.01.

little lower in these cows, but the difference reaches 1 per cent only at 5 yr. Only at 5 yr. is average chest depth as much as 0.5 cm. less in the cows in the present study.

Tables 3 and 4 give the intra-sire correlations and regressions of body measurements on inbreeding at the various ages. From these figures it is clear that inbreeding resulted in smaller size at all ages up to 2 yr. Although not all of the coefficients are statistically significant up to that age, the majority of them

are, and all are of negative sign and of similar magnitude. After about 2 yr., the inbreds grew the faster. At 5 yr. they were larger than non-inbreds, although not significantly so except in wither height and chest depth. The outbreds approached their mature size earlier. In the case of wither height, these cattle had reached 85 per cent of their 5-yr.-old height at 1 yr. Since wither height approached its mature size at the earliest age, it would be expected that any general effect of inbreeding in retarding early growth and prolonging later growth would show this reversal of sign at an earlier age for wither height than for the other measurements. The correlation coefficients reached their maximum negative values earliest with wither height and latest with heart girth and paunch girth. For all measurements except wither height, inbreeding resulted in significantly smaller size at 1 and 2 yr. In general, it appears that inbreeding slowed early growth rate but accelerated or prolonged it at later ages, so that mature size was unchanged or actually increased.

Tyler *et al.* (11), in three herds of Holsteins where inbreeding varied from 0 to 37 per cent, did not find that inbreeding had any significant effect on

TABLE 4

*Intra-sire regression coefficients of body measurements on inbreeding at various ages*

Age	No. sire groups	Wither height	Body length	Chest depth	Heart girth	Paunch girth
(yr.)						
0.5	19	-0.05	-0.12	-0.06 <sup>a</sup>	-0.14	-0.18
1	23	-0.10	-0.16 <sup>a</sup>	-0.09 <sup>a</sup>	-0.23 <sup>a</sup>	-0.29 <sup>a</sup>
2	24	-0.03	-0.15 <sup>d</sup>	-0.09 <sup>a</sup>	-0.29 <sup>a</sup>	-0.47 <sup>a</sup>
3	26	+0.11	-0.08	+0.05	0.00	-0.34
4	21	+0.24 <sup>b</sup>	-0.07	+0.09	+0.07	-0.27
5	20	+0.33 <sup>b</sup>	+0.06	+0.21 <sup>a</sup>	+0.32	+0.71

<sup>a</sup> 0.01 < P < 0.05.

<sup>b</sup> P < 0.01.

height at withers, circumference of shin bone or width at hips, either at 18 mo. or at maturity. They did find a significant decrease in heart girth at 18 mo. with increased inbreeding.

Bartlett *et al.* (2, 3, 9) found no difference between inbreds and outbreds for wither height but did find a slight advantage for outbreds in heart girth, both at 2 and 5 yr.

Baker *et al.* (1) found decreased wither height and heart girth with increased inbreeding.

*Type.* All animals were classified for type each year, resulting in most animals being classified several times. The official terms of excellent, very good, good plus, good, fair and poor were used, and it was intended that the classes would correspond to the official ones, except that each class was divided into three subclasses, low, medium and high, thus resulting in a total of 18 possible type classifications. Also, heifers were classified even before calving, which is not done in the official plan. Each subclass was given a numerical value starting with 0 for low poor and going to 18 for high excellent. The measure of type used for each cow was the average of all her ratings. To give more weight

to animals classified most often, this average was regressed towards the herd average according to the number of ratings included and using a repeatability of 0.4 for single type ratings (8). This makes the regressions a bit smaller numerically than if permanent type could have been classified without error. The average score for the herd was 7, which is equivalent to a classification of middle good. Data were available on the classification of 215 individuals whose average inbreeding was 4.6 per cent. They were sired by 37 different bulls and the differences in type between groups of daughters from different bulls were not statistically significant. The intra-sire correlation between inbreeding and type was  $-0.12$  and the regression of type on inbreeding was  $-0.04$ . For coefficients of this size to be statistically significant would require 300 observations, or 85 more than were available. However, if accepted at face value, they do indicate a tendency for poorer type as inbreeding increased. Using the regression coefficient obtained here, it would require an increase in inbreeding of 25–30 per cent to lower the average type classification one-third of a class; for example, from middle good to low good.

Bartlett *et al.* (2, 3), on 112 animals, found no significant effect of inbreeding on type.

*Production.* To determine the effect of inbreeding on production, butterfat records for 305-day lactations on a 3 times mature equivalent basis were used. The measure used for each cow was her lifetime average. The average of all of a cow's record is a more accurate indicator of her ability than is any single record, since random environmental errors will tend to cancel each other out of the averages. The average production of the 156 cows available for this phase of the study was 477 lb. Correlation coefficients and regressions of production on inbreeding were calculated on an intra-sire basis to help eliminate environmental changes, differences between sires, and effects of selection.

The average inbreeding coefficient was 4 per cent. The regression of production on inbreeding was  $-4.5$  lb. and the correlation coefficient was  $-0.23$ . The coefficients are statistically significant based on this volume of data. This regression coefficient indicates that in this herd an increase of 1 per cent in inbreeding was accompanied by an average decrease of 4.5 lb. of butterfat in a 10-mo. lactation.

Since selection was practiced for high production, the data were studied to determine if it were possible for selection to be strong enough to overbalance the decline expected as a result of inbreeding. From 1932 to 1942, the average inbreeding coefficient had increased from 1.6 per cent to 3.9 per cent, or an increase of 2.3 per cent. Providing there were no environmental changes or no selection for production, this increase in inbreeding would have been expected to lower average production by 10 lb. The yearly herd averages were adjusted for yearly changes in uncontrolled factors by a least squares procedure. The adjusted averages indicated that the average genetic merit of the herd for butterfat production had increased approximately 40 lb. Presumably, this is the increase left after deducting the decline caused by inbreeding. If there had been no inbreeding, this 40-lb. increase resulting from selection for high produc-



tion' would have been expected to be more nearly 50 lb. (40 lb. plus 10 lb. to compensate for the effect of inbreeding). This indicates that selection (mostly of bulls) was intense enough to more than counterbalance the effect of inbreeding. The above discussion is based on the assumption that environmental changes have been eliminated in the adjusted averages. Henderson (7) states that the least squares procedure used here for adjusting yearly herd averages, gives biased estimates when cows culled from the herd are either above or below the herd average. In this herd, during the period these records were made, little selection for production was possible among the cows since the test-and-slaughter policy was followed for controlling brucellosis and, in the early part of the period, reproductive rates were lower than in recent years because heifers were bred to calve at older ages and cows were not rebred as soon after calving as is now practiced. The average production of the cows leaving the herd usually was close to the herd average. Therefore, these estimates based on the least squares procedure should not have much bias from this cause.

These results of the effects of inbreeding are not in agreement with those reported by Woodward and Graves (12), in which they found no apparent effect on production. Tyler *et al.* (11) reported an average decrease of 74 lb. of milk and 2.3 lb. butterfat for each 1 per cent increase in inbreeding. Bartlett *et al.* (2, 4, 9) found that inbreds produced less milk and less total pounds butterfat than outbreds.

#### SUMMARY

The effect of mild inbreeding on size, type and production in a herd of Holstein-Friesian cattle was analyzed. The measures used were intra-sire correlations and regressions of the various traits on inbreeding.

The inbreeding of the individual itself resulted in an average decrease of about one-eighth of a pound in birth weight for each increase of 1 per cent in intensity of inbreeding. The inbreeding of the dam had a slightly smaller effect than this on the birth weight of the calf. Both values border on statistical significance.

For the five body measurements and weight at 6 mo. and over, an increase of 1 per cent in inbreeding never resulted in a decrease of more than 0.5 per cent and usually only about 0.1 per cent of the average of the respective measurement. The results indicate that the shape of the growth curve changes as the intensity of inbreeding changes. It appears that inbreeding slows the rate of growth at early ages but permits rapid growth to continue longer, so that mature size is not decreased and may even be increased. Although not all of the coefficients are significant, it is concluded that, in general, increased inbreeding resulted in a smaller size at least to 2 yr. and, for some of the later-maturing measurements, perhaps up to 4 yr. of age. A tendency for larger size with increased inbreeding was indicated at 5 yr.

Inbreeding had no certain effect on type ratings. A non-significant negative regression coefficient of type rating on inbreeding suggests a slight tendency for inbreeding to be detrimental to desired type.

Production decreased as inbreeding increased. The regression of butterfat production on inbreeding was  $-4.5$  lb. per 1 per cent inbreeding. This coefficient is significant at the 1 per cent level.

It was possible to practice enough selection for production, by using bulls from the best producing cows, to counterbalance the effect of inbreeding and to raise the genetic ability for butterfat production approximately 40 lb. during the 12 yr. studied. It appears that if a breeding plan is followed in which the increase in intensity of inbreeding is less than 2 per cent per generation, it should be possible to practice enough selection to counterbalance the decline in production expected to result from inbreeding.

Inbreeding did not result in the birth of any physically abnormal calves.

The numbers were too small and the intensity of inbreeding too low for any prediction of the effect of really intense inbreeding.

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## HORMONAL DEVELOPMENT OF THE MAMMARY GLAND OF DAIRY HEIFERS

J. F. SYKES AND T. R. WRENN

*Bureau of Dairy Industry, Agricultural Research Administration,  
United States Department of Agriculture*

The important activities of the secretions from the endocrine glands in the development of the mammary glands of several species have been indicated by a large body of experimental data. Recent reviews by Petersen (17), Turner (21) and Folley (2, 3) have summarized and integrated most of these studies. The predominant role of the ovarian hormones—the estrogens and progesterone—and of certain pituitary hormones has been emphasized.

In most species both estrogen and progesterone are necessary for duct and lobule-alveolar growth in intact animals. The guinea pig and possibly the monkey are exceptions in that complete mammary development can be induced by estrogen alone. In hypophysectomized animals, pituitary hormones (notably prolactin) are necessary, in addition to estrogen and progesterone, for mammary growth comparable to that developed during pregnancy.

Very few detailed studies have been conducted with the larger domestic animals. In 1941, Walker and Stanley (22) induced udder development and copious lactation in a castrate heifer by injecting steroid hormones. Subsequently, Folley and Malpress (5), Hammond, and Day (9) and Spriggs (19) on an extensive scale showed that marked udder development and copious lactation could be induced in unbred heifers by subcutaneous implantation of synthetic estrogen tablets. The copious lactation induced in many of these animals led to the obvious inference that complete mammary development had been induced by estrogen and that progesterone was not necessary for udder development in the bovine. No detailed histological examination of the udder tissues of these animals was made. Milk yield was the main criterion upon which estimates of the normality of mammary development were based.

The above studies were characterized by great variability in the gross appearance of the udder and in the lactation responses obtained, indicating that estrogen alone will not invariably induce udder development and lactation in the bovine. The lactation responses also usually were quantitatively smaller than might normally be expected. Therefore, on this basis also, it might be questioned whether completely normal udder development had taken place. The generally poor responses of freemartins (5, 9) to estrogen implants likewise would cast some doubt on the assumption that progesterone is unnecessary for complete mammary development in the bovine.

Results similar to the above have been obtained with the goat (6, 10, 14, 16). More detailed histological examination of the udder tissue was made in these

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studies. Lewis and Turner (10) noted that fairly complete lobule-alveolar development occurred in five goats after long continued treatment with diethylstilbestrol, and in three additional animals the lobules consisted of solid masses of cells. Mixner and Turner (16) noted that the alveoli that were developed by using estrogen alone were excessively large and exhibited a tendency to papillomatous folding, whereas the alveoli that were developed by using both estrogen and progesterone appeared more normal in structure. Folley (4) reported preliminary observations similar to those of Mixner and Turner (16), although Malpress (12), apparently referring to the same study (4), indicated there was no significant quantitative superiority of the alveolar tissue of animals treated with progesterone and estrogen over that of animals treated with estrogen only.

In all the studies noted above, fairly mature animals were used. In these studies, the effects of estrogen on mammary growth certainly were complicated to a variable and unknown extent by the experimental animals' own secretions. Even in the castrate studied by Walker and Stanley (22), the effects of the progesterone-like activity of adrenal-gland steroids cannot be ruled out. Such activity also may have been a factor in the mammary development noted by others in heifers (5, 9, 19), even though the ovaries in these heifers appeared to be inactive and, therefore, not producing appreciable quantities of progesterone. The extent to which the pituitary secretions of these animals participated in the response to estrogens also is unknown. Gardner and White (7) have suggested that prolactin sensitizes the mammary gland to estrogens. The secretions of the pituitary gland, either before or during treatment, therefore may have modified greatly the estrogenic effects noted in both the bovine and the goat.

Experimental work bearing on the problem of mammary development in the bovine does not permit definite conclusions concerning the hormonal mechanisms which may be involved. Little or no direct experimentation with various hormone combinations, including detailed examination of the tissues involved, has been conducted with the bovine species. The present study was initiated to investigate some of these factors in dairy cattle.

#### PROCEDURE

Eleven Holstein heifers were used in this study. They were given injections of stilbestrol or stilbestrol plus progesterone alone and in combination with a pituitary extract, as indicated in table 1. The pituitary extract used was prepared from fresh beef anterior lobe of the pituitary gland by the method de-

TABLE 1  
*Treatment of experimental heifers*

Group	No. of animals	Treatment
1	2	Stilbestrol
2	3	Stilbestrol plus progesterone
3	2	Stilbestrol plus pituitary extract
4	2	Stilbestrol plus progesterone plus pituitary extract
5	2	No treatment (controls)

scribed by Bergman and Turner (1) for their "initial extract." Several batches of this extract were prepared, dried, mixed and stored in the cold so that an homogeneous preparation could be used throughout the experimental period. Thirty mg. of the dried extract were injected into the animals of groups 3 and 4, three times weekly. The dried powder was dissolved in water and adjusted to pH 7.0 to 7.5 for injection purposes at weekly intervals.

Stilbestrol and progesterone were dissolved in olive oil and injected in this form. One mg. of stilbestrol was injected three times weekly in all the experimental animals until they reached 4 mo. of age. The dose of stilbestrol then was doubled and maintained at this level throughout the remainder of the experimental period. Progesterone was injected at the rate of 20 mg. three times weekly until 4 mo. of age and thereafter at the rate of 40 mg. three times weekly. The ratio of progesterone to stilbestrol injected was 20:1 throughout.

Injections were started when the calves were 1 mo. of age. In all instances, stilbestrol only was given for the first month and, in groups 2 to 4, inclusive, the additional hormones indicated in table 1 then were added to stilbestrol. This technique was employed in the hope that inhibition of pituitary and ovarian secretions would be produced by stilbestrol during the first month of injections and subsequently so that the observed effects on mammary growth at succeeding intervals could be related more directly to the activity of the injected hormones. When injections are started at this early age, the effects of the animals' ovarian secretions and the possible sensitizing or stimulating effects of pituitary secretions should be at a minimum, even when possible inhibition by stilbestrol is not considered.

Throughout the experiment the size of the udder was determined by palpation.<sup>1</sup> When the animals attained 5 mo. of age, half of the udder was removed for histological examination.<sup>2</sup> Injections were continued for an additional 4 mo., at which time the remaining half of the udder was removed at autopsy. Udder tissue was fixed in Bouin's fixative. Several blocks of tissues were obtained from various parts of the udder, and microtome sections were prepared from these for study after staining with hematoxylin and eosin. From these same areas larger and thicker sections were obtained and stained with Mayer's hemalum so that a somewhat detailed gross examination of gland architecture could be made.

In addition to udder tissue, the ovaries and the adrenal, thyroid and pituitary glands were obtained at autopsy and weighed. Bioassays for prolactin and gonadotrophic activity of the anterior lobe of the pituitary glands were made. Prolactin activity was determined by injecting pituitary tissue extracts intramuscularly into groups of 20 6-wk. old White Carnean squabs for 4 days. The crop glands of the pigeons were removed and weighed 96 hr. after the first injection. Results are expressed in units based on the table for conversion of crop sac weights to units as determined by Hall (8). These values may or may not be referable to international units but for comparative purposes this method of

<sup>1</sup> For these determinations the authors are indebted to W. W. Swett and his coworkers, C. A. Matthews and J. H. Book.

<sup>2</sup> The assistance of P. C. Underwood in these surgical procedures is gratefully acknowledged.

expression is adequate. Gonadotrophic activity was determined by injecting the pituitary extracts subcutaneously twice daily for 3 days into mice 21 to 28 days old. Groups of 10 mice were used for each dosage level. Uterine weights were determined 96 hr. after the first injection.

## RESULTS AND DISCUSSION

Udder development, as determined by palpation, was not increased by any of the hormone treatments over that observed in the control heifers. The grades assigned to udder development of the various animals are shown in table 2. The udders of the treated heifers (groups 1-4) in general appear to be slightly underdeveloped terminally as compared to the udders of the control heifers (group 5). This difference was not evident until the heifers were 4 to 5 mo. of age, and there was sufficient variation that it may not be significant. No significant differences between the groups of the treated heifers could be detected. Udder development as determined by palpation under these experimental conditions, however, is not a good measure of the type or amount of actual mammary-gland tissue which may

TABLE 2  
*Palpation grades<sup>a</sup> of udders of treated and control heifers*

Group	1		2			3		4		5	
Heifer	1	2	1	2	3	1	2	1	2	1	2
Age (mo.)											
1	5	4	5	5	—	6	—	5	5+	5	6
2	6+	6	7-	7-	6+	6+	7-	7	6+	7-	7-
3	7-	—	5	6+	7	5+	5	8	5+	6	4+
4	3+	6-	—	5	6+	4+	4+	—	6-	5+	4
5	3+	6	4	5-	6-	4	5	5+	6-	7-	5
6	—	—	—	—	—	3+	5+	5-	5	6	5
7	3+	—	5+	6+	—	—	—	—	—	—	—
8	4+	5-	—	—	4+	—	—	—	—	—	—
9	—	4+	4	5-	5-	4+	4	5-	6+	6+	6+

<sup>a</sup> The values refer to the numerical grades assigned in Swett's system of grading (20). (A grade of 5 is about average for the Beltsville herd.)

be present. The histological examinations indicated that the proportion of mammary tissue to fat was almost always greater in the treated than in the control heifers. The proportion of mammary tissue to fat also appeared to be greater at 5 mo. of age in the control heifers than it was at 9 mo.

No marked swelling of the udders with secretion occurred at any time in these heifers. Small amounts of secretion were present in the udders of all the treated heifers. The amount was small, never exceeding 100 ml., and, while it was milky in appearance, it did not appear to be milk of normal composition. Sufficient quantities for analysis were not obtained. No secretion was obtained from the control heifers.

Histological examination of the udder tissue showed that marked changes in mammary gland structure had occurred in all the treated heifers. The mammary glands of the control heifers consisted of ducts only, at both 5 and 9 mo. No alveoli were present at any stage. The treated animals, in addition to prolifera-

tion of ducts, showed considerable lobule-alveolar development. The relative amounts of duct and alveolar tissue varied, depending on the treatment and age of the animals.

Examination of the udder tissue obtained when the heifers were 5 mo. of age revealed marked differences in structure of the udders of the heifers which received steroid hormones only (stilbestrol or stilbestrol plus progesterone) as compared to those which received pituitary extract in addition to the steroid hormones. Definite alveolar development had occurred in the "steroid" heifers (groups 1 and 2) in all instances except one. With one exception, the udder tissue of the "pituitary" heifers (groups 3 and 4) consisted solely of duct tissue, although this was more extensive, as noted above, than in the control heifers. Fig. 1 illustrates typical differences observed in these two lots of animals.



FIG. 1. Typical mammary gland structure at 5 mo. of age.

A. From heifers injected with stilbestrol or stilbestrol plus progesterone. (X375)

B. From heifers injected with stilbestrol plus pituitary extract or stilbestrol plus progesterone plus pituitary extract. (X375)

Examination of the remaining udder tissue of these same heifers at 9 mo. showed that marked changes in development had occurred between the groups as contrasted to the development seen at 5 mo. The udders of the pituitary heifers (groups 3 and 4) consisted largely of well-developed lobules with mature-appearing alveoli. Some lobules contained excessively distended alveoli. Alveoli with thickened walls and with papillae projecting into the lumina were seen in some areas. The papillae were apparently similar to those noted by Mixner and Turner (16) in goats (fig. 2). The alveolar wall appeared to consist of a single layer of elongated cells and the papillae to be a result of folding of a portion of the alveolar wall. The structure of the udders of these heifers was in distinct contrast to that seen in the same heifers at 5 mo., when duct development only was an outstanding characteristic. In the succeeding 4 mo. very rapid lobule-



FIG. 2. Abnormal alveolar structure noted in certain areas.

A. Low power (X375) view showing thickened walls and papillomatous folding.

B. High power (X1770) showing elongated (columnar) cells of alveolar wall.

alveolar development had taken place and, in general, it exceeded that noted in the steroid heifers (groups 1 and 2) in spite of the fact that alveolar development was evident in these at 5 mo.

At 9 mo. further alveolar development had taken place in the steroid heifers over that seen at 5 mo. One udder was developed to a point comparable to that seen in any of the pituitary heifers, and in three others mature-appearing dis-



FIG. 3. Mammary gland structure at 9 mo. of age.

A. From heifers injected with stilbestrol plus pituitary extract or stilbestrol plus progesterone plus pituitary extract. (X375)

B. From heifers injected with stilbestrol or stilbestrol plus progesterone. (X375)



tended alveoli were more numerous than at 5 mo. of age, although many alveoli appeared to have advanced little beyond the terminal duct stage. The udder of one heifer in these groups at this time appeared definitely abnormal. It consisted almost entirely of solid cords of columnar cells, and the lobule formation was irregular and indistinct. Alveoli with thickened walls and papillae also were seen in the udders of these heifers but not as frequently as in the pituitary heifers. Fig. 3 illustrates the characteristic differences between the mammary structure of the steroid (groups 1 and 2) and the pituitary (groups 3 and 4) heifers as seen at 9 mo. of age.

The differences observed in structure of the glands of the steroid and pituitary heifers at the different ages are rather difficult to explain. The results obtained indicate that the pituitary extract increased the effects of the steroid hormones. The mammary glands of heifers receiving both steroid and pituitary hormones on the average appeared more nearly mature terminally, even though they were less mature earlier in development, than did the glands of heifers receiving only steroid hormones. The manner in which these effects occurred is more obscure, particularly when the differences noted at 5 and 9 mo. are considered.

It appears somewhat evident from this study that estrogen or estrogen plus progesterone will produce alveolar development at any stage, but a period of sensitization by pituitary hormones is necessary before their full effects may be realized. The occurrence of duct tissue only at 5 mo. of age in most of the heifers which received both steroids and pituitary extract would suggest that, when adequate hormonal stimuli (steroids plus pituitary hormones) are acting, the emphasis on udder development during the earlier stages is on extension and maturation of the more rudimentary duct tissue. At a later stage in development, providing that the requirements for maturation have been met, the emphasis would appear to shift to alveolar development. This is indicated by the rapid development of alveolar tissue in these heifers from 5 to 9 mo. of age. The failure of steroid hormones to produce alveolar development comparable to that produced by the combined action of steroids and pituitary extract could have been a result of inadequate sensitization or maturation of the rudimentary mammary tissues by pituitary hormones, in those cases where pituitary hormones were not injected.

A situation somewhat analogous to this can be visualized as occurring during normal development of the mammary gland of cattle. Up until the third or fourth month of pregnancy the udders of heifers consist solely of an extensive system of ducts. This rather prolonged period may be a period of extension and maturation of rudimentary tissues primarily under the influence of pituitary hormones, since the amounts of steroid hormones secreted during this interval are almost certainly very low. The rapid development of alveoli during the latter two-thirds of pregnancy could be a result of the previous maturation combined with the increased secretion of steroid hormones which occurs during pregnancy.

In the examination of udder tissue at both 5 and 9 mo. the authors have been unable to detect any effect of progesterone, either inhibitory or stimulatory, on the type or extent of development of the udder. These results are somewhat at variance with results reported by others in the goat (4, 12, 16). These results

may be due to the different estrogen-progesterone ratio used. Mixner and Turner (16) used 5  $\mu$ g. of stilbestrol to 1 mg. of progesterone whereas 50  $\mu$ g. of stilbestrol to 1 mg. of progesterone were used in this experiment. Mixner and Turner (15) have shown that a ratio of 25  $\mu$ g. of stilbestrol to 1 mg. of progesterone is adequate for mammary development in mice. These authors also note a rather wide range of estrogen-progesterone ratios which appear optimal for mammary growth. However, the ratio used here may not be optimal for the young bovine.

The data on weights of the endocrine glands which were obtained at autopsy

TABLE 3

*Endocrine gland—body weight ratios  $\times 100,000$  of experimental and control heifers*

Gland	Groups				
	1	2	3	4	5 (control)
Pituitary (whole)	0.56	0.64	0.85	0.85	0.90
Ovaries	1.22	0.96	1.64	3.29	5.99
Thyroid	5.40	7.04	8.37	8.86	8.36
Adrenals	5.00	4.97	5.77	6.19	4.61

indicate that, in certain instances at least, the injected hormones produced some inhibition of secretion in both the pituitary gland and the ovaries. These data are shown in table 3. Average values for each group are presented. The pituitary and thyroid glands and the ovaries were distinctly subnormal in the heifers (groups 1 and 2) that received steroid hormones only. The adrenals were slightly but not significantly larger than those of the control heifers. The reduced size of the ovaries and the thyroid gland apparently indicates that inhibition of both

TABLE 4

*Hormone content of anterior pituitary tissue of experimental and control heifers*

Group	Prolactin content (Units) <sup>a</sup>	Gonadotrophic activity (Mouse uterine weight) <sup>b</sup>
1	0	
2	1.0	
3	2.0	19.6
4	2.0	24.7
5	8.0	30.1

<sup>a</sup> Comparative values only—25.0 mg. (wet weight) of anterior pituitary tissue injected/bird.

<sup>b</sup> 16.0 mg. (wet weight) of anterior lobe tissue injected/mouse. (Control uterine weight = 24.7 mg.)

gonadotrophic and thyrotrophic hormone secretion by the pituitary gland had been produced in the heifers in these two groups. A similar inhibition of pituitary secretion probably was produced in the heifers of groups 3 and 4, as indicated by the subnormal ovarian size. The increased size of the adrenals over control values, the increase in the thyroid gland to normal values and the tendency for the ovaries to be somewhat larger than in groups 1 and 2 can be accounted for by the direct stimulating effect of the trophic hormones contained in the pituitary extract given to these heifers. The reduced size of the pituitary gland in

groups 1 and 2 also might suggest inhibition of activity of this gland. The essentially normal pituitary size observed in groups 3 and 4 is somewhat anomalous, however, since it might be expected that the injection of pituitary hormones would, in itself, cause a reduction in pituitary size.

The bioassay data also indicated that inhibition of pituitary-gland secretion occurred, if it is assumed that reduced hormone content of the gland represents reduced secretion and not merely exhaustion of the gland. These data are shown in table 4.

The data on gonadotrophic activity are quite unsatisfactory. No activity was detected in the pituitary tissue of any of the injected heifers when 16.0 mg. of this tissue were injected into immature mice. Only very slight activity was seen in the tissue from the control heifers. Sufficient tissue was not available to test the activity at higher dosage levels, and in groups 1 and 2 tissue was not available for testing at the 16.0-mg. level.

However, the pituitary tissue from the injected heifers definitely contained much less prolactin than did similar tissue from the control heifers. The very low prolactin content of these injected heifers differs markedly from the effects produced in other species by estrogen injections. Turner and his coworkers (11, 13, 18) have accumulated evidence which shows that the injection of estrogen increases the prolactin content of the pituitary glands of several species. The difference in the response of cattle to estrogen, as noted in the present study, may be a result of the prolonged period of injections and possible exhaustion of the pituitary gland or it may be a true species difference.

In general, the bioassay data and the data on endocrine gland size indicated that the effects on udder development were produced under conditions of reduced secretion of endogenous hormones. This was shown most definitely for the ovarian secretions, the data for pituitary secretions being suggestive. In any case, the rudimentary mammary tissue of these very young animals had been exposed to relatively little stimulation by hormones of endogenous origin before injections were started. These observations suggest that very young heifers may prove to be more suitable experimental subjects for determining hormonal effects on udder development than the more mature animals which have been used previously.

#### SUMMARY AND CONCLUSIONS

Accelerated mammary-gland development has been produced in very young dairy heifers by hormone injections.

The type of development and the sequence of developmental stages produced by stilbestrol or stilbestrol and progesterone were distinctly modified by the injection of a crude extract from the pituitary gland. Pituitary hormones accentuated the effects of these steroids on udder development. The udders developed as a result of injections of both steroids and pituitary extract appeared more mature structurally than the udders of heifers injected with steroids only.

In the dosages employed in this study, progesterone had no detectable effect on udder development.

Evidence is presented to indicate that the injections of the various hormones produced an inhibition of endogenous hormone production.

It is suggested that very young dairy heifers may be particularly suitable experimental subjects for determining the effects of the several hormonal factors which appear to participate in udder development of the bovine.

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## THE INFLUENCE OF CRACKED SOYBEANS AND OTHER FACTORS UPON FLAVOR OF MILK AND THE IODINE VALUE OF MILK FAT<sup>1, 2</sup>

J. B. FRYE, JR.,<sup>3</sup> C. Y. CANNON,<sup>4</sup> AND E. W. BIRD<sup>5</sup>

*Iowa State College, Ames*

The increased importance of soybeans as a protein supplement for dairy cows, and the feeling among certain workers in Iowa that the feeding of soybeans was contributing greatly to oxidized flavor in milk and cream suggested the need for more information regarding the effect of this feed on the flavor and quality of dairy products. It was thought advisable, therefore, to study the effect of cracked soybeans on the flavor of milk and the iodine value of the milk fat. During the course of the study, additional information was secured on the effect of production and age on the flavor of milk and the relationship of the iodine value of milk fat to environmental temperature.

Although feeds usually impart flavor to milk, the intensity of the flavor may be minimized when fed 2 to 4 hours before milking (3, 11, 15, 17, 30, 31, 32, 39, 40). Data published previously indicate that soybean feeding does not contribute particularly undesirable flavors to milk (4, 12, 21, 25, 26, 28, 34, 41).

The incidence of oxidized flavor in milk has been related to the oxidation of one or more of the lipids present in milk (8, 33, 37, 38) and the degree of saturation of the milk fat (14, 36). It has been shown that milk fat with the higher iodine value usually is more susceptible to oxidation (2, 6).

It is recognized that feeds may have a noticeable influence on the quality of butterfat produced (7, 10, 18, 20, 24). Peterson *et al.* (27) point out that the modification of milk fat by the diet occurs in opposition to the normal tendency of the gland to secrete a product of constant composition. Maynard *et al.* (23) noted that the maximum change was produced in the iodine value of milk fat

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<sup>3</sup> Present address: Dairy Department, Louisiana State University, Baton Rouge.

<sup>4</sup> Dairy Husbandry Subsection.

<sup>5</sup> Dairy Industry Section.

within 3 or 4 days when cows were fed rations containing fats having low and high iodine values.

Hilditch and Sleightholme (19) observed a general change in the composition of milk fat, largely in the oleic acid content, which they attributed mainly to "winter conditions" (*i.e.*, either the change from outdoor to indoor conditions or from grass to indoor rations or both), and probably also to seasonal changes of temperature. These workers concluded that the influence of added fat in the diet is minor compared to other causes. Likewise, Dean and Hilditch (9) indicate that the seasonal rise in the iodine value of the fat is rather abrupt with the change being completed within 2 or 3 weeks after the cows have been changed to pasture. Regan and Richardson (29) observed under controlled conditions that when external temperatures went above 80 to 85° F., dairy cows no longer were able to maintain heat balance and that alterations occurred in their milk including, among other things, an increase in the unsaturated compounds of the milk fat. These changes probably were the result of "hyperthermic under-nutrition."

#### EXPERIMENTAL PROCEDURE

*Feeding plan.* Twenty Holstein cows of the station herd were paired into two groups as equally as practicable. They were milked thrice daily and fed alfalfa hay *ad libitum* and grain mixtures (table 1), which contained cracked

TABLE 1  
*Grain mixtures used*

Ingredients	Mixture A	Mixture B
	(lb.)	(lb.)
Cracked corn . . . . .	400	400
Oats . . . . .	200	200
Wheat bran . . . . .	200	200
Linseed meal . . . . .	100	
Cracked soybeans . . . . .		100
Bonemeal . . . . .	18	18
Salt . . . . .	9	9

soybeans and linseed meal as the principal sources of protein, at the rate of 1 lb. grain for each 3 lb. of milk produced. All cows were fed the same ration (mixture A), which contained linseed meal, during a 70-day preliminary period. Then the animals in group 2 were changed to mixture B, which contained cracked soybeans, while those in group 1 continued to receive mixture A. These rations were reversed after 74 days and fed for the remainder of the trial (49 days).

*Collection and treatment of milk and cream samples.* Individual milk samples were taken in glass bottles and scored for flavor approximately 3 times each week. Composite samples were taken approximately twice each week during the last 3 mo. of the experiment. Usually the milk was scored within 6 hr. after being drawn from the cows. Representative aliquot samples of a day's milk yield from each group of cows were separated promptly after each milking at periodic

intervals and the cream cooled immediately to 40° F. by means of an ice-bath. The 24-hr. composite sample of cream was churned, and the butter melted, centrifuged, and the fat filtered at a temperature of 60–70° C. The butter oil was stored in glass sample jars at 10° C. until the analyses were completed. Iodine values of the butter oil were determined according to the Hanus method (1) approximately every 5 days during a period of about 6 mo.

#### RESULTS AND DISCUSSION

*Health of cows.* Two paired cows were dropped from the experiment because of mastitis and termination of lactation, respectively. The other cows were in excellent health for the duration of the study. The mean gains in live-weights for groups 1 and 2 were 42 and 29 lb., respectively (figure 1).

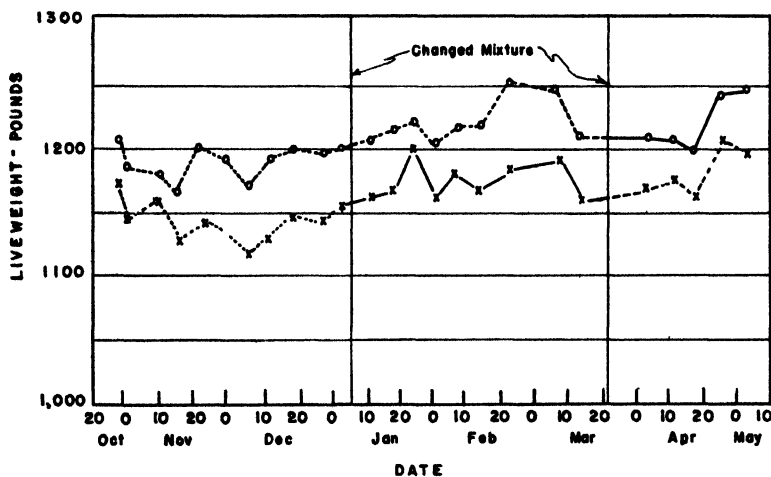


FIG. 1. Variation of live weights of the animals during the experiment. 0 = group 1; x = group 2; ---- = Mixture A (linseed meal); ——— = Mixture B (cracked soybeans).

*Relationship between yield, age of cow and flavor criticisms.* Plant operators often have suggested that one of the causes of off-flavored milk is the heavy feeding of cows to obtain high yields. To study this possible relationship, cows were fed so as to obtain their maximum production and individual samples were scored for flavor (table 2).

The differences in effect of mixtures A and B on milk flavor were minimized by the method of statistical analysis (between group correlations) (35). The correlation coefficients between total milk and fat production and percentage observations of (a) feedy, (b) oxidized, and (c) rancid flavors were non-significant (table 3).

This is in agreement with the work of Stebnitz and Sommer (36) and Hening and Dahlberg (17), who found that the level of feeding has no effect upon the quality and flavor of milk. On the other hand, Henderson *et al.* (16) indicate that a high level of feeding increases the susceptibility of milk to development of



TABLE 2

*Data used to study the relationship between age, milk yield, fat yield and various observations of off-flavor*

Cow no.	Age	Production during trial		Total times milk was off-flavor	Percentage distribution of off-flavor observations among flavor classifications			
		Milk	Fat		Feedys <sup>a</sup>	Flat and feedy	Oxidized	Rancid
	(days)	(lb.)	(lb.)					
1497	1424	8892	268	36	72.2	8.33	11.11	13.89
1528	2800	6357	246	31	36.5		3.23	45.16
1544	2052	6913	227	36	88.9	13.89	2.78	8.33
1557	1781	6083	252	31	9.7	3.23	3.23	87.10
1587	1167	6000	213	36	88.9	30.56	2.78	8.33
1599	1163	8515	253	30	50.0	6.67	33.33	16.67
1614	1195	7308	254	34	67.6	14.71		2.94
1708	834	9116	306	36	66.8		16.92	5.56
1712	817	5627	184	30	50.0	30.00	43.33	6.67
1384	1786	7386	240	35	82.9	2.87		11.42
1806	1404	7786	280	36	66.6	22.21	13.89	16.91
1518	1322	7026	246	37	97.3	18.92		2.70
1539	2865	8159	284	29	51.8	3.45	10.34	20.69
1553	1591	5193	178	35	94.3	28.57	5.72	
1561	1262	8052	278	36	91.6	19.44	5.56	2.78
1581	1210	7966	254	35	57.2	2.86	31.43	11.42
1696	924	6470	240	30	60.0	30.00	30.00	10.00
1713	789	5822	211	30	33.4	26.67	60.00	6.67

<sup>a</sup> Includes those samples with "flat and feedy" criticism.

oxidized flavor. MacCurdy and Trout (22) report that when cows were fed a given quantity of silage the feed flavor was more intense in milk from cows of least production. Their data indicate that high producers, such as the ones producing approximately 40 lb. daily used in this study, produce milk with less feed flavor than low producers. In this respect, the two experiments are in agreement.

TABLE 3

*Statistical summary of data used in flavor study*

Correlation determined	$\bar{x}$	$Sx^2$	$\bar{y}$	$Sy^2$	$Sxy$	Correlation coefficient
Milk <sup>a</sup> —feedy flavor	7148	23,232,096	64.7	9151	-3711	-0.00
Milk—oxidized flavor	7148	"	15.2	5032	-36,526	-0.11
Milk—rancid flavor	7148	"	15.4	6488	-67,373	-0.17
Milk—flat flavor	"	"	14.6	2102	-134,690	-0.61**
Fat <sup>a</sup> —feedy flavor	245	19,122	64.7	9151	-1148	-0.08
Fat—oxidized flavor	"	"	15.2	5032	-2498	-0.26
Fat—rancid flavor	"	"	15.4	6488	1902	0.17
Fat—flat flavor	"	"	14.6	2102	-4128	-0.65**
Age <sup>b</sup> —feedy flavor	1466	6,257,912	64.7	9151	-30,334	-0.04
Age—oxidized flavor	"	"	15.2	5032	-94,849	-0.53*
Age—rancid flavor	"	"	15.4	6488	-93,056	-0.46
Age—flat flavor	"	"	14.0	2102	-59,322	-0.52*

<sup>a</sup> Refers to yield.

<sup>b</sup> Refers to age of cow.

\* = Significant at 5% level.

\*\* = Significant at 1% level.

Significant negative correlation coefficients (table 3) of  $-0.61$ ,  $-0.65$ , and  $-0.52$  between flat flavor and (a) milk yield, (b) fat yield, and (c) age, respectively, indicate that older cows and higher producers are less likely to produce milk having a flat flavor. Usually, flat flavor and fat in the milk are associated together, the milk with the lower butterfat having a "flatter" flavor. The authors wish to emphasize that the total fat yields, and not fat percentages, were used in these comparisons. The correlation coefficient between age and the percentage observations of feedy flavor was non-significant.

The correlation coefficient ( $-0.46$ ) between age and the occurrence of rancid flavor was  $0.004$  less than the significant point. The correlation coefficient ( $-0.53$ ) between age and the occurrence of oxidized flavor was significant, in fact, almost highly significant. Insofar as these data are concerned, the individual sample data indicate that rancid and oxidized flavors occurred more frequently in the milks of the younger cows. These data are in contrast with those of Guthrie and Bruecker (13) who found no apparent relationship between age and the incidence of oxidized flavor. However, they are in agreement with those of other workers (5, 42) who have indicated that young cows are more likely to produce milk with a rancid or oxidized flavor than are older ones. In a 6-mo. study of milk samples from 138 cows, Corbett and Tracy (5) found that 2- and 3-yr. old cows gave milk (1 p.p.m. Cu added) which developed oxidized flavor to a greater degree than did that from older cows. Why young cows, especially first calf heifers, should have a greater tendency to produce milk more susceptible to the development of oxidized flavor than older cows is not known. These data do not mean necessarily that the same cow produces milk less susceptible to the development of oxidized or rancid flavors as she grows older. Perhaps the explanation is that many of the younger animals, which produce milk susceptible to the development of rancid or oxidized flavor, may be culled from the herd for one reason or another and leave cows less likely to produce milk susceptible to the development of these flavors.

*Flavor criticisms of composite milk samples.* Certain cows in group 1, fed linseed meal (table 4) during the first experimental period, consistently produced milk with a rancid flavor. When the milk from these cows was excluded from the composite milk sample, the flavor was improved considerably. The incidence of rancid milk produced by these cows diminished after they were fed cracked soybeans in the latter part of the experiment. On the other hand, the cows in group 2, (fed cracked soybeans) produced milk quite free from rancidity and there was little change in the quality of the milk after the cows were switched to mixture A. These data do not mean necessarily that the feed contributed to the incidence of rancidity in the milk. They may mean that certain cows are much more susceptible to the feed than others. Factors other than feed may have been responsible for the high incidence of the cows in group 1; the individuality of the cow probably played an important role. It is concluded that, in addition to factors that normally are considered, the selection of cows to be used in flavor studies should be based on the flavors of the milk they produce when fed a common ration.

It is noteworthy that the oxidized flavor usually characteristic of composite milk samples was absent, despite its presence in many of the individual samples. These changes may indicate that the oxidation-reduction potential of the mixed sample *may be* lowered to a point at which the oxidation is reversed or other flavors "cover" the oxidized flavor.

TABLE 4  
*Flavor scores and criticisms of composite milk samples\**

Sampling date	Group 1		Group 2	
	Score	Criticism	Score	Criticism
	Mixture A (linseed meal)		Mixture B (soybeans)	
Jan. 30	20.0	Feedy & slightly rancid	21.0	Feedy
Jan. 31	20.0	Feedy & slightly rancid	21.0	Feedy
Feb. 3	20.0	Slightly rancid	21.5	Feedy
Feb. 5	21.0	Feedy	19.5	Rancid
Feb. 8	21.0	Feedy	21.5	Feedy
Feb. 10	19.5	Rancid	21.5	Feedy
Feb. 13	19.0	Rancid	21.5	Feedy
Feb. 14	19.0	Rancid	21.0	Feedy
<sup>b</sup> Feb. 25	21.5	Flat & feedy	21.5	Flat & feedy
<sup>b</sup> Feb. 26	21.0	Flat & feedy	21.0	Flat & feedy
<sup>b</sup> Feb. 28	21.0	Feedy	21.0	Feedy
Mar. 3	18.0	Rancid	20.0	Feedy
<sup>b</sup> Mar. 4	20.5	Slightly rancid	21.5	Flat & feedy
<sup>b</sup> Mar. 5	20.0	Feedy	21.5	Flat & feedy
Mar. 6	19.0	Rancid	21.0	Flat & feedy
<sup>b</sup> Mar. 10	21.0	Feedy	21.0	Feedy
Mar. 18	18.0	Rancid	21.0	Feedy
	Mixture B		Mixture A	
Mar. 21	19.5	Rancid & feedy	21.0	Flat & feedy
Apr. 10	20.0	Rancid & feedy	21.5	Feedy
Apr. 11	21.0	Feedy	21.0	Feedy
Apr. 18	20.5	Feedy	21.5	Feedy
Apr. 21	20.0	Rancid & feedy	21.0	Feedy
Apr. 24	20.5	Feedy	21.5	Feedy
Apr. 25	20.0	Feedy	21.0	Feedy
May 2	20.0	Rancid	21.5	Feedy
May 5	20.5	Feedy	21.0	Feedy
May 9	21.0	Feedy	21.5	Feedy

\* Feedy and flat flavors were not considered undesirable.

<sup>b</sup> When milk from cows gave rancid milk was excluded from the sampling, the composite sample was not rancid.

*Iodine value of milk fat.* The iodine value of the milk fat was used to determine when the full effect of the feed on the milk fat was reached. It was supposed that the iodine values of the milk fat would soon stabilize themselves when the cows were fed a common ration. However, it became apparent as the experiment progressed that the iodine values did not stabilize as rapidly as had been supposed by some workers, and that it was necessary to determine the time required to stabilize the iodine values before making a "cross-over" of feeds. This determination is more easily and more accurately accomplished when the

iodine value is determined frequently, as was done in this experiment. Changes in fat composition made when the iodine value was determined only 2 or 3 times each month might fail to reveal many of the fluctuations of the iodine value and indicate a false stabilization. Since the literature did not disclose trends in iodine values of milk fat except in relatively short-time feeding trials, a new objective, determination of the long-time effect of feed on the iodine values of milk fat, was injected into the experiment. Consequently there was no change

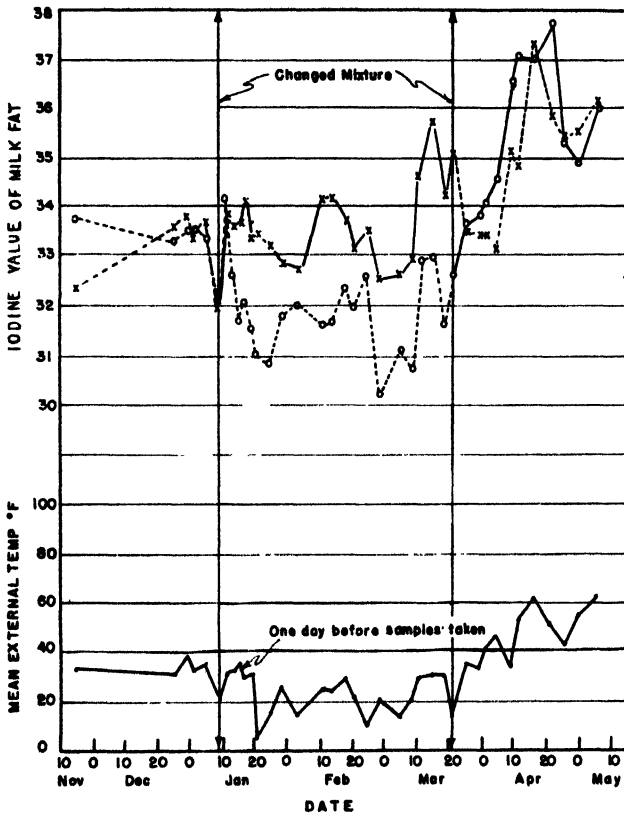


FIG. 2. The iodine values of milk fat and the mean external temperatures one day before that on which the milk fat was obtained. ---- = Mixture A (linseed meal); — = Mixture B (cracked soybeans).

in the feeding program after the end of the preliminary period until the latter part of the trial. The iodine values (figure 2), which were determined weekly, were larger in the milk fat produced by the cows in group 2 fed on mixture B (soybeans) than that of group 1 which received mixture A. The full effect of the soybeans on the iodine value of the milk fat seemed to be attained in about 15 days after the cows were changed to this ration. The differences in the iodine values of the milk fats of the two groups were fairly constant until the rations

were reversed, when the positions of the resultant iodine values soon were reversed. In approximately 15 days, a fairly constant difference in the values was established and the difference was smaller than during the previous period. This small difference was not maintained long, for at slightly past the mid-point of the period the curves behaved randomly and crossed twice. This would indicate that factors other than feed were operating to influence the trend and magnitude of the iodine values. Of these factors, changes in temperature may have played an important role.

The fluctuations of the iodine values and the mean external temperature recorded 1 day prior to taking of samples (fig. 2) were somewhat the same. As the temperature dropped to a low level during the winter months, the iodine

TABLE 5  
*Statistical summary of data used in study of mean external temperature and iodine value relationship*

Correlations between temperature and iodine values <sup>a</sup>	$\bar{x}$	$Sx^2$	$\bar{y}$	$Sy^2$	$Sxy$	Correlation coefficient (r)	Regression coefficient (b)	T-test of regression coefficient
Temp. same day—I <sub>2</sub> no. and grp. 1	30.1	7473	33.14	124	696	0.72**	0.0931	6.42**
Temp. 1 day before—I <sub>2</sub> no. and grp. 1	31.3	6405	33.17	133	755	0.82**	0.1178	8.60**
Temp. 2 days before—I <sub>2</sub> no. and grp. 1	29.9	5888	33.17	133	651	0.74**	0.1106	6.54**
Temp. same day—I <sub>2</sub> no. and grp. 2	30.1	7473	33.94	51	317	0.51**	0.0424	3.69**
Temp. 1 day before—I <sub>2</sub> no. and grp. 2	31.3	6405	33.97	56	397	0.66*	0.0592	5.10*
Temp. 2 days before—I <sub>2</sub> no. and grp. 2	29.9	5888	33.97	56	412	0.72*	0.0699	6.19*

<sup>a</sup> Mean external temperatures were recorded (1) the same day, (2) one day before, and (3) two days before the milk fat samples were taken.

\* = Significant at 5% level.

\*\* = Significant at 1% level.

values likewise decreased in value. As the temperature rose with the approach of spring, the iodine values also increased. Correlation coefficients (table 5) between the temperature on the same day, 1 day before, and 2 days before the samples were taken and iodine values were all highly significant. These data indicate that temperature changes show their greatest relationship with butterfat composition 24 to 48 hr. later. Whether or not this correlation is wholly a function of temperature is difficult to state. Changes in hormonal activity with advancement in lactation and pregnancy may have played a role in the iodine value fluctuations. Further investigation of the correlation between the iodine value of milk fat and temperature should be undertaken.

## SUMMARY

Twenty Holstein cows of the station herd were used to study the influence of cracked soybeans, level of production and age upon the flavor of milk and the relationship of the iodine number of butterfat to feed and temperature changes.

In addition to the usual factors considered in selecting cows for flavor studies, the flavor of the milk produced by the cows while being fed a common ration should be considered.

There was no indication that cracked soybeans produced an undesirable flavor in milk when constituting approximately 11 per cent of the concentrate mixture.

The extreme fluctuations in iodine value with chronological time and the randomness of the last half of the last feeding period suggest that control groups should be carried continuously on each feed when fat composition changes are studied.

The correlation coefficients between milk and fat yields, and the occurrence of feedy, oxidized and rancid flavors in the milk were non-significant.

Highly significant negative correlations between milk ( $-0.61$ ) and fat ( $-0.65$ ) yields and the occurrence of flat flavor indicate that, as the total milk and fat production increase, the tendency for the production of milk having a flat flavor decreases. Likewise, a significant negative correlation ( $-0.52$ ) was found between age and the occurrence of flat flavor.

The correlation coefficient between age and the occurrence of feedy flavor was non-significant.

The correlation coefficient ( $-0.46$ ) between age and the occurrence of rancid flavor was only 0.004 below the significant point. Possibly rancid flavors have a greater tendency to develop in the milk of younger cows.

A significant negative correlation coefficient ( $-0.53$ ) was obtained between age and the occurrence of oxidized flavor.

The maximum effect of a feed on fat composition, as measured by the iodine value, may require at least 15 days.

A close relationship may exist between mean external temperature and the iodine value of the milk fat produced.

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## A COMPARISON OF VARIOUS SEMEN DILUTERS IN MAINTAINING MOTILITY OF BOVINE SPERMATOZOA<sup>1</sup>

GLENN R. PURSLEY, H. A. HERMAN, MAURICE DICKENSHEET AND R. E. WATERS

*Department of Dairy Husbandry, University of Missouri, Columbia*

One of the major problems in the artificial insemination of dairy cattle is the improvement of diluters for the successful storage of semen. Experiments were conducted by Phillips and Spitzer (9) to study the effects of certain protein, lipid and carbohydrate compounds, as well as certain bacteriostatic agents, upon the livability of bull spermatozoa. The egg yolk-citrate diluter reported by Salisbury *et al.* (10, 12) has been used widely in artificial insemination. Swanson (13) reported 3 per cent sodium citrate to be optimum for semen dilution and storage. Increased fertility has been reported by the addition of 300 mg. per cent of sulfanilamide to the egg yolk-citrate by Knodt and Salisbury (4). Salisbury and Knodt (11) and Salisbury *et al.* (12).

Satisfactory conception rates were obtained by use of the liquid and tablet diluters produced by the Ortho Pharmaceutical Co. in comparison with egg yolk-phosphate, egg yolk-citrate and egg yolk-citrate-sulfanilamide diluters (1, 3).

The objective of this study was to compare the efficiency of several diluters now in general use and to attempt to develop an improved diluter by (a) substituting sodium citrate for  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{PO}_4$  as the buffering system in the pabulum diluter suggested by Phillips and Spitzer (9), (b) substituting egg-yolk for asolectin in the pabulum diluter to determine if the "protective factors" for spermatozoa are present in comparable quantities and (c) substituting dried egg yolk for fresh egg yolk as a possible means of simplifying laboratory procedure in artificial insemination associations.

In addition, a pabulum diluter supplied by the Ortho Pharmaceutical Co. and control samples of undiluted semen were studied.

### EXPERIMENTAL

Semen from ten dairy bulls from the Missouri Station dairy herd totaling 117 samples was used in the comparison of diluters. Each sample was divided in aliquot portions and used in each of the diluters tested. The makeup of these diluters, except the Ortho diluter, is given in table 1. There were 117 comparisons of the egg yolk-citrate diluter and the six pabulum diluters. An undiluted control sample semen was maintained when available and there were 75 comparisons of the undiluted samples and the Ortho liquid diluter.

Immediately after collection of the semen, the vial was placed in a thermos bottle inside a glass tube which was surrounded with tap water at 70° F. About

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TABLE 1  
*Formula and pH of each diluter*

	EYC	I	II	III	IV	V	VI	OL
Glucose (g.)		1.2	1.2	1.2	1.2	1.2	1.2	
Galactose (g.)		0.4	0.4	0.4	0.4	0.4	0.4	
KH <sub>2</sub> PO <sub>4</sub> (g.)				0.4	0.4	0.4		
Na <sub>2</sub> HPO <sub>4</sub> (g.)				1.58	1.58	1.58		
Na Citrate (g.)	3.1	3.53	3.53				3.53	
Asolectin (g.)		4.0	4.0	4.0	4.0			
Gum Acacia (g.)		6.0	6.0	6.0	6.0	6.0	6.0	
Sulfanilamide (mg.)		66	66	60	66	66	66	
Water (redistilled over glass) to final vol. (ml.)	100	200	200	200	200	200	200	
Egg yolk (ml.)	100		20		20	20		
pH of diluter	6.69	6.5	6.6	6.8	6.87	6.92	6.66	6.3

EYC = egg yolk citrate; I, II, IV, V and VI are modifications of the synthetic pabulum diluter; III = synthetic pabulum diluter. OL = Commercial Ortho Liquid diluter.

30 min. elapsed between the time of collection, examination and dilution of the semen. Equal volumes of semen and diluter were used in all dilutions. After dilution the vials containing the diluted semen were stored in a refrigerator which maintained an average temperature of 40° F. This procedure in preliminary trials was found satisfactory for lowering the temperature of the diluted semen approximately 1° F. per min.

Motility ratings on the stored spermatozoa were made by daily microscopic examinations.

Since Herman and Swanson (14) and Margolin *et al.* (5) found a highly significant correlation between the length of time a sample retained a "2" motility rating in storage (approximately 20 to 50 per cent motile spermatozoa) and conception rate, the methods suggested by Herman and Swanson (2) and later revised by Swanson and Herman (14) were used.

## RESULTS

The average number of hours each diluent maintained a motility of "2" was computed from the data and is presented in table 2. An analysis of variance was

TABLE 2  
*Hours "2 motility" was maintained in semen under storage conditions*

Diluent <sup>a</sup>	Hr. <sup>b</sup>
EYC	174.2 ± 44.5
I	159.9 ± 45.9
II	157.7 ± 50.0
III	177 ± 36.3
IV	172.7 ± 47.3
V	182.7 ± 47.6
VI	190.6 ± 43.8
Ortho liquid	109.3 ± 33.2
Undiluted	82.7 ± 22.9

<sup>a</sup> Composition of diluents given in table 1.

<sup>b</sup> Necessary difference =  $t \sqrt{2(\sigma^2 \text{ ejaculates} + \text{remainder})} = 14.7 \text{ hr.}$

made on the six pabulum diluents and the egg yolk-citrate diluent. Due to the unequal number of samples studied for the Ortho liquid diluter and the undiluted samples, analysis of variance was not made, but these samples were compared by statistically analyzing the difference of the means. The results are presented in table 3. The six pabulum diluents and the egg yolk-citrate diluent were signifi-

TABLE 3  
*Analysis of variance between diluters and ejaculates*

Source	D/F	Variance	Mean square
Total	818	4,233,499	
Diluents	6	1,559,970	259,995*
Ejaculates	116	269,592	2,324
Remainder	696	2,403,937	3,454

\* Highly significant ( $p = 0.01$ ).

cantly superior to the Ortho liquid and undiluted semen at the 1 per cent level.

#### DISCUSSION

The livability of spermatozoa ranged from 109.3 to 190.6 hr. in the various diluents studied. Since all diluters were made up to have approximately the same osmotic pressure and, with exception of the Ortho liquid diluter, agreed closely in pH, the variations in livability of the spermatozoa under storage conditions apparently were due to physiological differences brought about by the chemical make-up of the medium.

The results of the six pabulum diluters and the egg yolk-citrate diluter were studied for statistical significance by analysis of variance. The Ortho liquid diluter and the undiluted samples, which were in unequal numbers, were compared with the pabulum diluents by a statistical analysis of the difference of the means. A difference of 14.7 hr. was required for significance at the 1 per cent level.

In comparing diluents I and II with diluent III, which is the original synthetic pabulum suggested by Phillips and Spitzer (9), indications are that the replacement of  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  with sodium citrate is detrimental to spermatozoa under storage conditions and that the addition of egg yolk fails to improve the storage capacity of these diluents.

In comparing diluents I and II with diluent VI, it appears that sodium citrate and asolectin in combination apparently are antagonistic; since diluent VI is significantly superior at the 1 per cent level. In addition to the increase in mean storage time provided by diluent VI, the spermatozoa are more easily observed under the microscope when asolectin is replaced by egg yolk.

All diluents studied were superior to the Ortho liquid diluent and the undiluted samples, although the Ortho diluent gave a mean of 26.6 hr. greater livability than the undiluted semen.

## SUMMARY

A comparison of the egg yolk-citrate diluent and the synthetic pabulum diluent with several modifications for preserving semen was made.

A formula is presented for the preparation of a diluent for use in the artificial insemination of dairy cattle which appears, under conditions of these experiments, to support significantly greater livability of spermatozoa than previously suggested diluters.

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## SOME EFFECTS OF HYPERTONIC AND HYPOTONIC SOLUTIONS ON THE LIVABILITY AND MORPHOLOGY OF BOVINE SPERMATOZOA<sup>1</sup>

GLENN R. PURSLEY AND H. A. HERMAN

*Department Dairy Husbandry, University of Missouri, Columbia*

Studies reported to date on diluting fluids for bovine semen have presented only limited information concerning the effects of varying freezing point depressions of diluents on spermatozoa livability and morphology. Osmotic swelling and distension of the head of mammalian spermatozoa generally has not been observed, according to Anderson (1). He further states, "The tails in hypertonic solutions show irregular zig-zag bends, while in hypotonic solutions, especially in distilled water, the tails are curled in rings." Anderson (1) also calls attention to the statement by Milovanov that mammalian sperm have retained the ability of adaptation to changes in osmotic pressure to a certain extent. The work of Roemmele who found that the osmotic pressure in terms of the freezing point depression of bull semen was  $-0.62^{\circ}\text{C}$ . with a range of from  $-0.54$  to  $-0.73^{\circ}\text{C}$ ., and also Bernstein and Sergin who report an average freezing point depression of  $-0.609^{\circ}\text{C}$ . with a range of  $-0.53$  to  $-0.65^{\circ}\text{C}$ . is cited by Anderson (1). Salisbury *et al.* (7) found the freezing point depression of bull semen to be  $-0.653^{\circ}\text{C}$ . A positive and significant correlation coefficient of 0.33 between spermatozoa count and the freezing point depression also was reported. As the spermatozoa count decreased the magnitude of the freezing point depression increased.

Salisbury *et al.* (8) obtained satisfactory results using a M/15 sodium citrate solution with egg yolk. Later, it was indicated by these workers that a M/7.5 solution was being used. Salisbury and Knodt (9) presented a revised formula, using 3.6 per cent sodium citrate dihydrate in the egg yolk-citrate diluent. Salisbury *et al.* (7) found that fresh normal bull semen has the same osmotic pressure as cattle blood and they recommend 2.9 per cent sodium citrate for the diluent. Swanson (10) found that 3 per cent sodium citrate dihydrate was superior to other concentrations tried. Bratton *et al.* (3) obtained similar results using 2.9 and 3.6 per cent egg yolk-citrate. Both of these concentrations contained 300 mg. per cent of sulfanilamide.

Swanson (10) found that 5 per cent sodium citrate had an immediate adverse effect on spermatozoa motility and that 1 per cent citrate diluent was tolerated better than 5 per cent. The 1 per cent solution failed to maintain satisfactory motility and resulted in a high proportion of coiled-tail spermatozoa which moved only backwards or in circles. He observed that bovine spermatozoa are more sensitive to hypertonic solutions of sodium citrate than to hypotonic solutions, and offers as explanation the fact that as the semen ages, lactic acid increases, re-

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sulting in an increase in osmotic pressure which is likely counteracted by a hypotonic solution but only aggravates an already incompatible condition in a hypertonic solution.

In view of the limited information on the effect of varying osmotic pressure on bovine spermatozoa, an investigation of some of the effects of hypertonic and hypotonic solutions on spermatozoa livability and morphology was made. The objectives were: (a) to determine the effect of hypertonic and hypotonic solutions on the spermatozoa cell membrane, head shape, and dimensions; (b) the effects of solutions of varying osmotic pressure on livability of spermatozoa in storage; (c) a comparison of the freezing point depressions of various semen diluents now in use; and (d) to determine if the addition of 300 mg. per cent of sulfanilamide to egg yolk-citrate would markedly increase the livability of spermatozoa under storage conditions.

#### EXPERIMENTAL

The freezing point depression of semen and the various diluents was measured by means of a standard cryoscope which depended upon the evaporation of ether for cooling. A calibrated thermometer measuring temperatures in  $0.01^{\circ}$  C. was used for all recordings. The apparatus was standardized using varying levels of C. P. sucrose solutions, distilled water and milk. The freezing point of milk was assumed to be  $-0.55^{\circ}$  C. The necessary correction factors were applied in computing all freezing point depressions.

Twenty-two semen samples obtained from five sires in the Missouri Station dairy herd were used in this study. Two ejaculates from each sire were pooled in order to have sufficient volume for measurement of the freezing point depression. The sodium citrate concentrations used in the various diluting solutions are presented in table 1. Isotonic and 12.5 to 100 per cent hypo- and hypertonic diluting solutions were studied.

The livability of spermatozoa in the various strength solutions under storage conditions at  $40^{\circ}$  F. was measured by microscopic examination. Motility ratings were made immediately after dilution and then at 24-hr. intervals. The samples were retained as long as 20 per cent of the spermatozoa remained progressively motile. This is the lower limit of a "2" motility as suggested by Herman and Swanson (5). All motility ratings were made in intervals of five percentage units.

The morphology of spermatozoa in different strength solutions was studied by use of stained slides. Rose Bengal was used as a stain, according to the method of Herman and Swanson (5). Photomicrographs of the stained spermatozoa were made at a magnification of  $430\times$ . The ocular in the camera contained a micrometer divided into squares with a calibration of  $0.1\times 0.1$  mm. Slides  $3''\times 4''$  for use in a projection lantern were made from the photomicrographs of spermatozoa in iso-, hypo- and hypertonic solutions. Measurements for spermatozoan head size were made by projecting the slides on a screen at a fixed distance. The micrometer squares when projected on the screen were  $50''\times 50''$ , therefore 1 in. on the screen was equal to  $2\mu$  on the slide.

TABLE 1

*Make-up of diluents, freezing point depressions, pH and livability of spermatozoa*

Diluent	$\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$	Egg yolk	F.P. $\Delta$	pH	Livability spermatozoa <sup>a</sup>	S.D.
	(per 100 ml. $\text{H}_2\text{O}$ )	(ml.)	(°C.)		(Mean hr.)	(hr.)
1 <sup>a</sup>	6.2	100	-0.93	6.78	10.9	36
2 <sup>b</sup>	6.2		-1.05	7.37	0.0	
3	5.425	100	-0.85	6.77	38.7	51
4	5.425		-0.94	7.36	0.0	
5	4.65	100	-0.74	6.74	95.5	81
6	4.65		-0.84	7.38	1.1	
7	3.875	100	-0.67	6.71	131.1	68.5
8	3.875		-0.69	7.46	9.8	
9	3.488	100	-0.61	6.69	149.5	68.3
10	3.488		-0.61	7.43	13.1	
11	3.1	100	-0.59	6.76	163.6	62
12	3.1		-0.57	7.42	18.5	
13	2.713	100	-0.47	6.72	177.8	67
14	2.713		-0.48	7.46	10.1	
15	2.325	100	-0.44	6.75	173.7	69
16	2.325		-0.44	7.47	20.7	
17	1.55	100	-0.33	6.67	188.5	58
18	1.55		-0.29	7.50	9.3	66
19	0.78	100	-0.22	6.53	162.5	73.5
20	0.78		-0.19	7.50	0.0	
EYC <sup>c</sup>					176.4	63.6
EYC SA <sup>d</sup>					190.9	82.7

<sup>a</sup> Odd numbers = Na citrate + equal amount of egg yolk.<sup>b</sup> Even numbers = Na citrate buffer only.<sup>c</sup> EYC = egg yolk citrate.<sup>d</sup> EYC SA = egg yolk citrate + 300 mg% sulfanilamide.<sup>e</sup> Necessary difference for significance at 5% level = 29.7 hr.

## RESULTS

Data on the mean number of hours each diluent maintained a minimum of 20 per cent motile spermatozoa during storage at 40° F. are presented in table 1. The protective or favorable influence of egg yolk in prolonging livability of spermatozoa in solutions varying considerably from isotonicity is quite apparent,

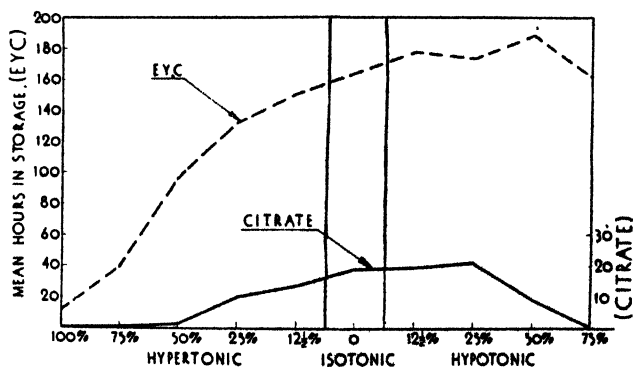


FIG. 1. The effect of hypertonic and hypotonic solutions on livability of spermatozoa stored at 40° F.

and is illustrated in figure 1. An analysis of variance was made on the egg yolk-citrate samples that were stored sufficiently long for comparisons to be made. This analysis is presented in table 2.

TABLE 2  
*Analysis of variance between diluents and ejaculates*

Source	D/F	Variance	Mean sq.
Total	219	1,216,221	
Diluents	9	745,701	82,855*
Ejaculates	21	166,450	7,926
Remainder	189	304,070	161

\* Significant ( $P < .05$ ) necessary difference 29.7 hr.

An analysis was made of the percentages of progressively motile spermatozoa at intervals during the first 7 days of storage. These averages are given in table 3.

TABLE 3  
*Average per cent live spermatozoa during storage for each motility rating period above 20 per cent motile*

Storage (hr.)	% live spermatozoa in diluent no.								
	5 <sup>a</sup>	7	9	11	13	15	17	19	EYC EYCSA
1	27	40	50	56	56	53	54	37	55 51
24	24	35	45	50	50	46	50	36	49 42
48	20	25	32	41	43	41	43	32	43 34
72		19	23	33	36	33	37	24	36 28
96			20	28	28	28	33	22	31 23
120				24	25	24	27	18	25 19
144				19	20	19	22		19
168							18		

<sup>a</sup> Diluent no. as given in table 1.

The spermatozoan head dimensions in the different solutions, as measured by projection on a screen, are presented in table 4. There were no measurable

TABLE 4  
*Spermatozoa head dimensions in hypertonic and hypotonic solutions<sup>a</sup>*

Freezing point depression	Head width times length
(°C.)	(μ)
-1.05	4.9 × 9.7
-0.94	5.2 × 9.7
-0.84	5.1 × 9.8
-0.69	5.1 × 9.9
-0.61	5.2 × 10.0
-0.57	5.2 × 10.2
-0.48	5.0 × 10.1
-0.44	5.2 × 10.0
-0.29	5.1 × 10.1
-0.19	5.4 × 10.3

<sup>a</sup> Average spermatozoa head dimensions =  $5.1 \times 10.0 \mu$ .



changes in the head dimensions of spermatozoa in the hypertonic and hypotonic solutions studied.

Pictures of a field of spermatozoa in each of the hypertonic and hypotonic solutions are presented in figures 2 and 3. Abnormalities in spermatozoan morphology seem to occur only in the stronger hypertonic and the stronger hypotonic solutions, and are characteristically coiled tails.

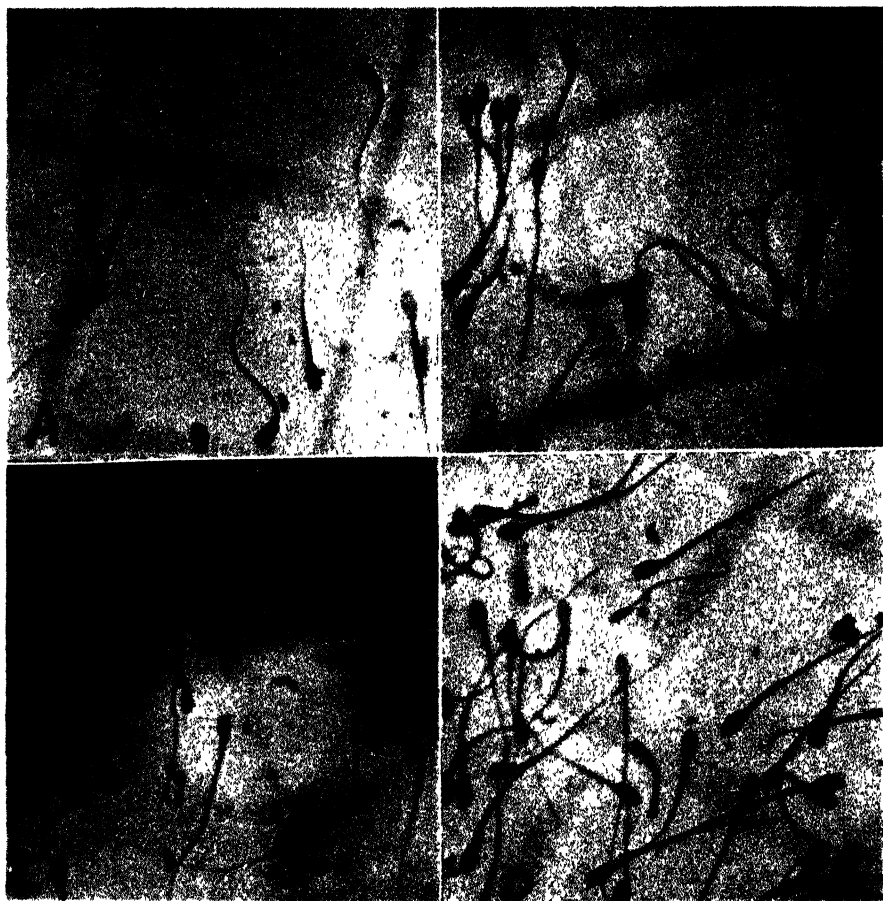


FIG. 2. Photomicrographs of spermatozoa in isotonic and hypertonic solutions. A—Freezing point  $-0.57^{\circ}\text{C}$ . (Isotonic); B—Freezing point  $-0.61^{\circ}\text{C}$ .; C—Freezing point  $-0.94^{\circ}\text{C}$ .; D—Freezing point  $-0.104^{\circ}\text{C}$ .

#### DISCUSSION

There is a gradual decline in motility of spermatozoa stored at  $40^{\circ}\text{F}$ . in solutions with increasing hypertonicity, reaching the low of 10.9 hr. in the strongest (100 per cent) hypertonic solution used. This solution has a freezing point depression of  $-1.05^{\circ}\text{C}$ . From the data presented in table 1, it would seem that

a freezing point depression of  $-0.69^{\circ}\text{C}$ . would be the uppermost limit in formulating diluters. This conclusion is in agreement with the work of other investigators (1, 3, 7) who found that the freezing point depression of bull semen ranged from  $-0.54$  to  $-0.73^{\circ}\text{C}$ .

The effect of hypotonic solutions, as used in this study, on spermatozoan livability are in striking contrast to the results with hypertonic solutions. The livability of spermatozoa in the weakest hypotonic solution used (freezing point

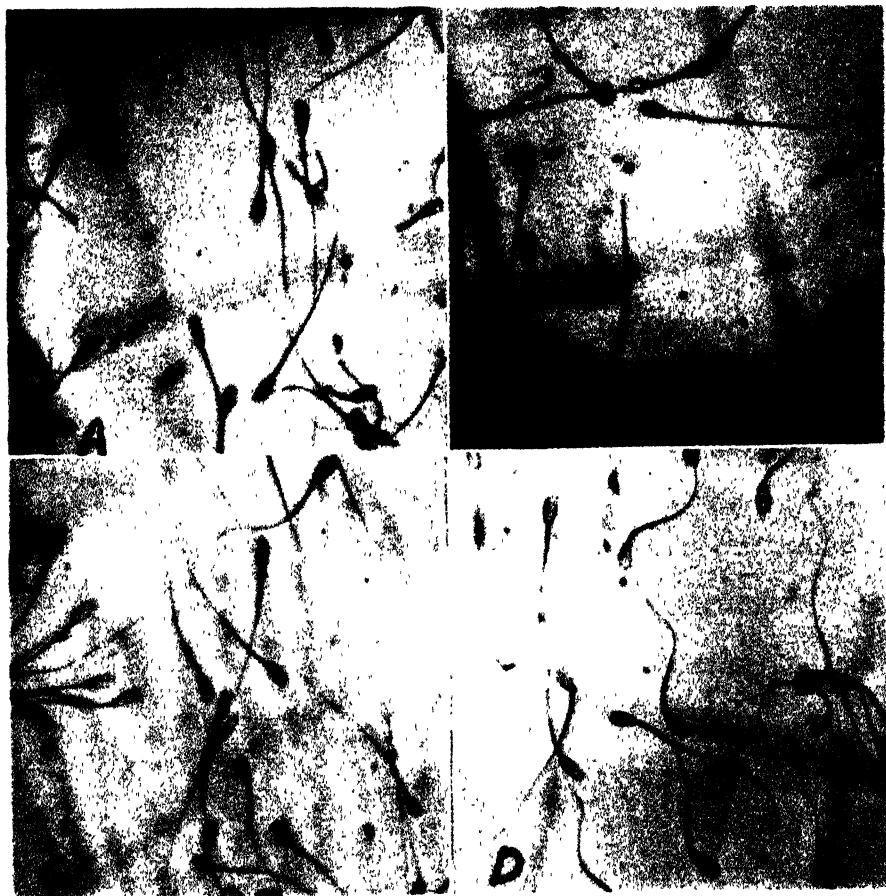


FIG. 3. Photomicrographs of spermatozoa in hypotonic solutions. A—Freezing point  $-0.19^{\circ}\text{C}$ .; B—Freezing point  $-0.29^{\circ}\text{C}$ .; C—Freezing point  $-0.44^{\circ}\text{C}$ .; D—Freezing point  $-0.48^{\circ}\text{C}$ .

depression  $-0.19^{\circ}\text{C}$ .) was comparable to that obtained in the isotonic solutions with a freezing point depression of  $-0.57^{\circ}\text{C}$ . The distribution of the egg yolk in hypotonic solutions with freezing point depressions of  $-0.19$  to  $-0.29^{\circ}\text{C}$ . seemed to be more even than in stronger sodium citrate solutions and may be a

factor in preserving longer livability. In the 3 per cent sodium citrate-egg yolk solution as well as all other hyper- and hypotonic solutions, except the two mentioned above, there was a tendency for the egg yolk components to settle out.

Disregarding the two weakest hypotonic solutions discussed above, it would appear that diluents with freezing points ranging from  $-0.44$  to  $-0.61^{\circ}\text{C}$ . would be optimal for spermatozoa survival, since there is no significant difference in any of the diluents studied within this range. In terms of buffer solutions used for artificial insemination this would require 2.3 to 3.5 per cent sodium citrate dihydrate per 100 ml.

Since there were no differences observable in the head size of spermatozoa in the hypertonic and hypotonic solutions, the conclusion that bovine spermatozoa have a very low permeability for salts must be drawn. Abnormalities consisting of a low percentage of coiled tails were observed in the stronger hypertonic and the weaker hypotonic solutions, but in these cases the head dimensions of the spermatozoa were not affected in measureable amounts.

Anderson (2) reported the dimensions of the average bull spermatozoa head to be  $5\mu$  long and  $2\mu$  wide. Savage and Williams (6), in studying the head length variability of bovine spermatozoa and its application to the determination of fertility, found the mean head length to range from  $9.4$  to  $9.6\mu$ . In this study the average spermatozoa head dimension was found to be  $5.1 \times 10\mu$  and agrees closely with the results obtained by Savage and Williams (6) in their study of fertility.

#### SUMMARY

1. A study was made of the effects of several hypertonic and hypotonic solutions on livability and morphology of spermatozoa. The freezing point depressions ranged from  $-0.19^{\circ}$  to  $-1.05^{\circ}\text{C}$ .

2. The optimum range for spermatozoa survival was  $-0.44$  to  $-0.61^{\circ}\text{C}$ . in terms of freezing point depressions or 2.3 to 3.5 per cent in terms of sodium citrate dihydrate concentration.

3. There appeared to be little difference in the spermatozoa head dimensions in the various hypertonic and hypotonic solutions. Coiled tails were observed in the two strongest hypotonic solutions and in the strongest hypertonic solution.

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# THE COMPARATIVE VALUE OF LADINO CLOVER, BIRDSFOOT TREFOIL, TIMOTHY AND ALFALFA HAYS FOR YIELD AND QUALITY OF MILK

J. K. LOOSLI, VLADIMIR N. KRUKOVSKY, G. P. LOFGREEN<sup>1</sup> AND R. B. MUSGRAVE  
*Cornell University, Ithaca, N. Y.<sup>2</sup>*

For several years experiments have been in progress at Cornell University to obtain more complete data on the value for milk production of certain hay crops, adapted to the northeastern United States. As a part of this program, studies were made during the winters of 1947-48 and 1948-49 to compare birdsfoot trefoil (*Lotus corniculatus*) and ladino clover hay with timothy and alfalfa hay harvested at two stages of maturity. While birdsfoot trefoil and ladino clover are used most commonly as pasture plants in mixtures with the grasses, it sometimes is desirable to harvest them as hay or hay-crop silage. It appeared desirable to learn more about the value of hay made from ladino clover and birdsfoot trefoil. Timothy and alfalfa hay also were studied for comparative purposes.

## EXPERIMENT I

The early-cut timothy hay was mowed on June 16 and 21, 1947, before the plants had headed out. The late-cut timothy was harvested on August 7, 1947, when the seeds were becoming hard. The early-cut timothy hay yielded 3,498 lb. and the late-cut 4,078 lb. of dry matter per acre. Yield data were not obtained for the other hays. The early-cut timothy was no. 1 and the late-cut, no. 3. The legume hays used were second cutting. This was selected because it was possible to obtain a stand freer from weeds and grasses than with first cutting. Alfalfa was made at two stages of maturity as with timothy. The early cutting was made when the first blossoms appeared and the late stage was past full bloom with some seed pods present. Both hays were fine-stemmed, leafy and bright in color and were graded as no. 1 leafy alfalfa. The ladino clover was cut in July before blossoms had appeared. The birdsfoot trefoil was cut during September and October when the forage was in the pre-bloom stage and approximately 10 in. high.

After a period of wilting in the sun, the hays were picked up with a field chopper, cut into approximately 4-in. lengths and blown into racks which were connected to a drier in such a manner that heated air could be blown through the chopped hay. Artificial drying was used to prevent curing variables owing to weather damage. During the curing process some molding was encountered in the ladino clover hay because the green material packed together so that it was difficult to force the heated air through it. These molded spots were sorted

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<sup>1</sup> Present address: Animal Husbandry Department, Univ. of Cal., Davis.

<sup>2</sup> Departments of Animal Husbandry, Dairy Industry and Agronomy.

out before the hay was fed, but it is likely that the spoilage reduced somewhat the carotene content of the hay. The chemical composition of the hays used is shown in table 1.

TABLE 1  
*The chemical composition of the hays*

Type of hay	Composition						
	Moisture	Protein	Fat	Fiber	N.F.E.	Ash	Carotene
	(%)	(%)	(%)	(%)	(%)	(%)	(mg./lb.)
<i>Experiment I</i>							
Alfalfa, early-cut	9.9	17.6	1.7	30.2	33.5	7.0	33.7
Alfalfa, late-cut	7.8	14.0	1.9	30.6	39.0	6.7	34.0
Birdsfoot trefoil	9.5	13.1	2.1	28.4	41.0	6.0	33.0
Ladino clover	11.6	21.4	1.4	18.6	36.8	10.4	16.2
Timothy, early-cut	9.2	9.3	2.0	31.9	41.0	6.7	22.0
Timothy, late-cut	10.0	4.9	1.3	37.9	40.8	5.2	2.3
<i>Experiment II</i>							
Alfalfa	7.5	12.3	2.2	26.3	45.5	6.1	55.4
Birdsfoot trefoil	7.9	16.8	2.2	25.7	41.0	6.4	54.0
Ladino clover	7.6	17.7	2.3	24.8	39.8	7.8	81.3
Timothy	7.0	6.0	1.7	35.7	46.3	3.3	11.8

The Holstein cows used in the study were removed from pasture and allowed a 1-wk. adjustment period before they were placed upon the experiment. The grain mixture fed was adjusted at the start of each period to the rate of 1 lb. for each 5 lb. of 4 per cent fat-corrected milk (FCM). Hay was fed three times daily in liberal amounts so that each cow refused 1 to 3 lb. per day. Corn silage was fed at a constant rate of 1.5 lb. for each 100 lb. of bodyweight. Records were kept of the feed allowed and refused. The milk production was recorded at each milking and an aliquot sample taken of four milkings each week for fat test by the Babcock method. At the end of each experimental period, samples of milk were obtained for tests on flavor, stability and vitamin content.

Fifteen cows were used in the lactation experiment. The plan of study involved an incomplete block design<sup>a</sup> set up with four experimental periods of 5 wk. each. In the plan of the experiment, ten observations were to be made on each of the six types of experimental hay. However, the quantity of ladino clover was insufficient to complete the study and this hay was not fed during the fourth experimental period.

## RESULTS

The average daily intake of the various hays, the actual and adjusted production of 4 per cent FCM and the gain or loss of body weight are shown in table 2.

From these data it is clear that the late-cut timothy was appreciably less palatable than were the other hays, since only 11 lb. were consumed per day, as compared with 24 to 28 lb. of the other types of hay. In every case, when a cow was shifted from another type of hay to late-cut timothy, her intake immediately fell and she declined in milk production very rapidly, so that within 3 wk. the

<sup>a</sup> For the design see *Statistical Tables for Biological, Agricultural, and Medical Research*, R. A. Fischer and F. Yates. Oliver and Boyd, Ltd., London. Pp. 14 and 57. 1943.

daily FCM yield was only approximately 70 per cent of the initial production even though her grain and silage allowances were kept constant. No such declines in the intake of hay or in milk production were observed in changing among the other types of hay. The effect upon production clearly is evident from the average data (table 2), appreciably less FCM being produced on late-cut timothy than on any other hay. Furthermore, the cows uniformly lost weight when late-cut timothy was fed, whereas, in almost every instance, they maintained their weight or gained when the other hays were fed. As an average, the cows lost 2.07 lb. in weight each day on late-cut timothy whereas they gained from 0.68 to 0.90 lb. per day on the other types of hay.

Part of the difference in milk yield can be accounted for by the fact that the cows used differed in initial production and in the rate of decline with advancing lactation. The experimental design makes it possible to adjust for these differences. When the adjustments are made, a more accurate estimate is obtained of the comparative value of the hays for milk production. Such an estimate is presented in the last column of table 2.

TABLE 2

*The average daily hay intakes, production of 4% fat-corrected milk and change in weight of the cows (expt. I)*

Type of hay	Av. wt. of cows	Average daily intake			Gain in wt.	FCM <sup>a</sup>	Adjusted FCM
		Hay	Silage	Grain			
	(lb.)				(lb.)	(lb.)	(lb.)
Alfalfa, early-cut	1305	25.1	18.1	8.7	0.73	34.3	36.0
Alfalfa, late-cut	1179	28.1	15.6	7.1	0.89	33.2	28.1
Birdsfoot trefoil	1145	27.1	16.9	7.2	0.68	33.9	33.6
Ladino clover	1244	24.5	17.1	8.7	0.73	39.0	34.2
Timothy, early-cut	1200	23.7	16.9	8.0	0.90	34.0	32.7
Timothy, late-cut	1002	11.2	17.2	7.6	-2.07	24.8	24.2

<sup>a</sup> 4% fat-corrected milk.

These data still overestimate the value of the late-cut timothy hay for milk production, since the cows lost body weight on this hay and undoubtedly converted body energy into milk. In order to evaluate the importance of this change in weight, an estimate was made of the productive T.D.N. value of the hay by allowing for the maintenance requirements of the cows, for the changes in weight which occurred during the experiment, for the actual milk secreted and for the other feeds consumed. In making this estimate, it was assumed that 8 lb. of T.D.N. were required for the maintenance of a 1,000-lb. cow. T.D.N. values of 75 per cent for grain and 19 per cent for silage were used in all comparisons. It was assumed that 0.32 lb. of T.D.N. were required for each pound of FCM produced, that each pound gain in body weight required 2.53 lb. of T.D.N. and that each pound of body weight lost was equivalent to 2.73 lb. (Knott *et al.*, 1). The estimated productive T.D.N. value of the hays, based on these data, are shown in the last column of table 3. Also there are presented in table 3 the average digestion coefficients and the T.D.N. (total digestible nu-

TABLE 3

*The average digestibility and total digestible nutrient value of the hays<sup>a</sup>*

Type of hay	Digestion coefficients				N.F.E.	Total digestible nutrients	Productive T.D.N. of the hays <sup>b</sup>
	Dry matter	Crude protein	Ether extract	Crude fiber			
	(%)	(%)	(%)	(%)	(%)	(lb./100 lb.)	(lb./100 lb.)
<i>Experiment I</i>							
Alfalfa, early-cut	62.1	68.2	20.0	60.7	64.5	52.7	53.3 <sup>c</sup>
Birdsfoot trefoil	64.3	68.5	31.7	54.1	72.0	54.7	48.6
Ladino clover	73.1	78.8	15.8	65.3	77.1	57.9	60.4
Timothy, early-cut	65.0	61.4	41.6	65.6	67.1	55.9	59.1
Timothy, late-cut	45.6	24.4	23.0	45.3	49.7	39.2	11.6
<i>Experiment II</i>							
Birdsfoot trefoil	64.8	75.6	31.8	55.7	72.0	59.0	49.0
Ladino clover	70.0	74.2	37.2	65.2	76.8	61.8	43.5

<sup>a</sup> Four animals were used with each type of hay.<sup>b</sup> Estimated from the lactation study.<sup>c</sup> Late-cut alfalfa showed a value of 52.0 for T.D.N.

trients) for the different hays as determined with yearling wethers. Four wethers were fed each hay in turn as the only feed in a digestion study. Sheep were used because insufficient hay was available for the use of dairy animals after completion of the lactation study. Unfortunately, the late-cut alfalfa was used completely in the lactation study and its digestibility was not obtained.

Ladino clover hay showed higher digestion coefficients (table 3) than any of the other hays for dry matter, protein and nitrogen-free extract, and it was highest in T.D.N. by both measures used. All nutrients in the late-cut timothy hay, except ether extract, were decidedly less digestible than in the other hays; the T.D.N. value also was inferior.

Samples of hay were taken at each period in the lactation study for carotene analyses. The average values obtained are shown in table 1. The carotene, vitamin A and total tocopherol content of the milk fat samples taken at the end of each experimental period are shown in table 4. From these data it is clear

TABLE 4

*The vitamin content and the stability of the milk. (expt. I)*

Type of hay	Milk fat concentration (γ/g.) of:			Samples showing oxidized flavor
	Carotene	Vit. A	Tocopherols	
				(%)
Alfalfa, early-cut	4.9	5.6	23.7	0
Alfalfa, late-cut	6.4	6.0	23.8	0
Birdsfoot trefoil	7.4	7.0	28.3	0
Ladino clover	4.3	4.1	17.7	57
Timothy, early-cut	4.7	5.2	22.0	0
Timothy, late-cut	2.6	4.7	19.0	0

that the late-cut timothy contained less carotene than any of the other hays and that ladino clover was next to the lowest. In general, the carotene and vitamin A content of the milk tended to follow the order of the carotene content of the



hay samples except for ladino clover. Also, the milk produced during the ladino clover periods was significantly lower in tocopherol content than when the other hays were fed. Tests made for flavor and stability showed that off-flavor milk occurred only during periods when ladino clover hay was fed. The oxidized flavor in the milk appears to be correlated with the lower tocopherol content (Whiting *et al.*, 3; Krukovsky *et al.*, 2), suggesting that the content of this vitamin in the ration may be related to the stability of milk. Milk produced on birdsfoot trefoil was highest in carotene, vitamin A and tocopherol content and it had excellent keeping qualities.

It appeared possible that changes during the curing of ladino clover which resulted in the development of slight molding may be related to the low carotene content of the hay and the low vitamin content of the milk when this hay was fed. In experiment II, special attention was given to curing the ladino clover and to testing the vitamin content and stability of the milk.

TABLE 5

*The U. S. grade, botanical composition and green color index of the hays*

Designation of hay	U. S. Grade of hay	Botanical composition <sup>a</sup>	Green color (%)
Alfalfa <sup>b</sup>	A. no. 1 grass hay	32% legumes	64
First cutting	B. no. 3 mixed hay	59% grasses 9% weeds	29
Birdsfoot trefoil	Not under U. S. standards	47% legumes 52% grasses 1% weeds	52
Ladino clover	U. S. no. 1 extra	70% legumes	63
Second cutting	green mixed hay	20% grasses 10% weeds	
Timothy	U. S. no. 1 timothy	(Not sorted)	49
First cutting	light grass mixed		

<sup>a</sup> Based on weighed separations made at the time of cutting.

<sup>b</sup> Approximately  $\frac{1}{3}$  of the alfalfa had browned slightly during curing and green and brown samples were graded separately.

#### EXPERIMENT II

Four types of hay were compared in the second study during the fall and winter of 1948-49. These included alfalfa, timothy, birdsfoot trefoil and ladino clover. Only one stage of maturity was available because of a shortage of material, and all of the legumes were heavily mixed with grasses (table 5), although they had been planted as pure stands for this experiment only 2 yr. before. The timothy hay was cut in the bloom stage and field-cured. The alfalfa was in early bloom but the grasses in the mixture were not headed out at the time of cutting. The ladino clover and birdsfoot trefoil were cut before bloom, somewhat earlier than generally would be recommended for hay. The U. S. grades, the botanical composition as estimated by sorts at cutting and the green color rating of the hays are shown in table 5.

The amounts of hay produced were less than planned and, as a result, only

four cows could be used in the lactation study. The  $4 \times 4$  Latin square design was employed with each period 4 wk. in length. In order to increase the accuracy and control of the test, a group of cows was studied for 6 wk. on pasture before they were started on the hay experiment. From the data collected, four cows were selected which were very uniform in production, stage of lactation and gestation, body size and age, and these cows were allotted at random to the treatments. The feeding and management was similar to the first experiment. The chemical composition of the feeds is shown in table 1. The values shown are averages of samples taken and analyzed during the different periods of the lactation trial. The management of the cows and collection of samples were the same as for experiment I. The data on feed intakes and milk production are shown in table 6.

TABLE 6

*The average daily feed intakes, yield of 4% fat-corrected milk and gains in body weight of the cows. (expt. II)*

Type of hay	Average daily intake			Average daily		Productive T.D.N. of the hays
	Hay	Grain	Corn silage	Gain in wt.	FCM	
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb./100 lb.)
Alfalfa	37.6	8.0	20.0	0.82	35.9	44.2
Birdsfoot trefoil	34.6	8.0	20.0	0.88	36.4	49.0
Ladino clover	34.7	8.0	20.0	0.35	36.9	43.5
Timothy	31.2	8.0	20.8	-0.28	30.9	35.7

As an average, the same amounts of corn silage and grain were fed with all the hays. Alfalfa was slightly more palatable than the other hays and timothy hay was appreciably less palatable. Approximately equal amounts of milk were produced on the three legume hays, but when timothy hay was fed the cows produced significantly less 4 per cent fat-corrected milk (odds, 99:1). As an average, 3 wk. after the cows were changed from a legume to timothy hay they were producing only 80 per cent as much FCM as they were giving at the start of the period. Furthermore, the cows lost weight on timothy but gained on the other hays. This lowered production on timothy undoubtedly was due to the later stage of maturity of the timothy at harvest, since in experiment I, fully as much milk was produced on early-cut timothy hay as on early-cut legume hay. The estimated productive T.D.N. value of the timothy hay was appreciably less than for the legumes. Digestion studies were made with the birdsfoot trefoil and ladino clover hays using four lambs. The digestion coefficients obtained (table 3) are in close agreement with those from experiment I.

Samples of milk and blood were obtained from the cows each week during the pasture period and also during the hay-feeding experiment. The samples were analyzed for carotene, vitamin A and total tocopherols. The stability of the milk fat was measured during a 7-day storage period with added copper. The vitamin content of the milk fat and the blood plasma and the relative stability of the milk during the last week of each period is summarized in table 7.

Although the ladino clover was highest in carotene content (table 1), the blood plasma and milk fat were lowest in vitamin A value when ladino clover hay was fed. The tocopherol content of the milk fat and the stability of the milk were lowest on ladino clover and highest on birdsfoot trefoil, in agreement with the data from experiment I. The differences were less marked, however, than in the first test. It appears that the dilution of the legumes with grasses, in comparison with the clear legumes fed in the first study, may explain the smaller relative effects on milk quality, but it is important that the differences persisted in spite of the presence of the grasses. The vitamin content of the milk fat and its relationship to the roughage fed and to the stability of milk is illustrated in fig. 1. In general, the milk showed a striking tendency to develop oxidized flavors during storage when the milk fat contained less than 20  $\gamma$  of tocopherols per gram of fat.

TABLE 7

*The carotene, vitamin A and tocopherol content of the blood plasma and milk, and the stability of the milk. (expt. II)*

Type of forage	Blood plasma concentrations ( $\gamma$ /100 ml.) of:			Milk fat concentration ( $\gamma$ /100 g.) of:			Samples showing oxidized flavor
	Caro- tene	Vita- min A	Toco- pherol	Caro- tene	Vita- min A	Toco- pherol	
							(%)
<i>Pasture</i>							
Ladino-orchard grass	1,372	22	695	886	1,066	2,934	50
Birdsfoot trefoil- blue grass	1,375	56	975	982	1,024	4,225	0
<i>Hay</i>							
Alfalfa	800	70	546	486	733	2,116	40
Birdsfoot trefoil	654	68	466	436	593	2,290	33
Ladino clover	691	56	452	434	434	1,951	100
Timothy	631	64	491	410	652	2,155	50

## DISCUSSION

These experiments illustrate the high value for milk production of early-cut, well-cured hay. The excellent quality hays successfully maintained milk yield even when very limited amounts of corn silage and grain mixture were fed. In these tests the hay furnished fully two thirds of the T.D.N. required. By contrast, in earlier studies (4) when late-cut timothy hay was fed, cows failed to maintain normal rates of milk production when the grain-feeding rate was reduced so that the cows had to obtain more than 25 per cent of their T.D.N. requirements from the hay.

The legume hays, alfalfa, ladino clover and birdsfoot trefoil, when cut in early bloom and properly cured, were approximately equal in value for milk production, on the basis of the two studies conducted. Timothy hay cut at heading stage or earlier appeared equal in value to the legumes, but when timothy was cut in bloom or later it was markedly inferior in feeding value.

Both experiments agree with other studies (Krukovsky *et al.*, 5) in showing

that the type of roughage fed may have a marked effect upon the stability of the milk produced. The results suggest that ladino clover, whether fed as hay or pasture, may predispose the milk to the early development of oxidized flavors. Other types of high quality roughage, especially birdsfoot trefoil, appear to increase the stability of milk. These implications of the influence of roughages on milk quality are very important and warrant further research. Because of the importance of ladino clover as a pasture and silage crop, if further studies confirm these preliminary indications that ladino may influence milk quality adversely, means should be sought to overcome the defect by selecting improved varieties, growing plant mixtures or feeding richer sources of tocopherols.

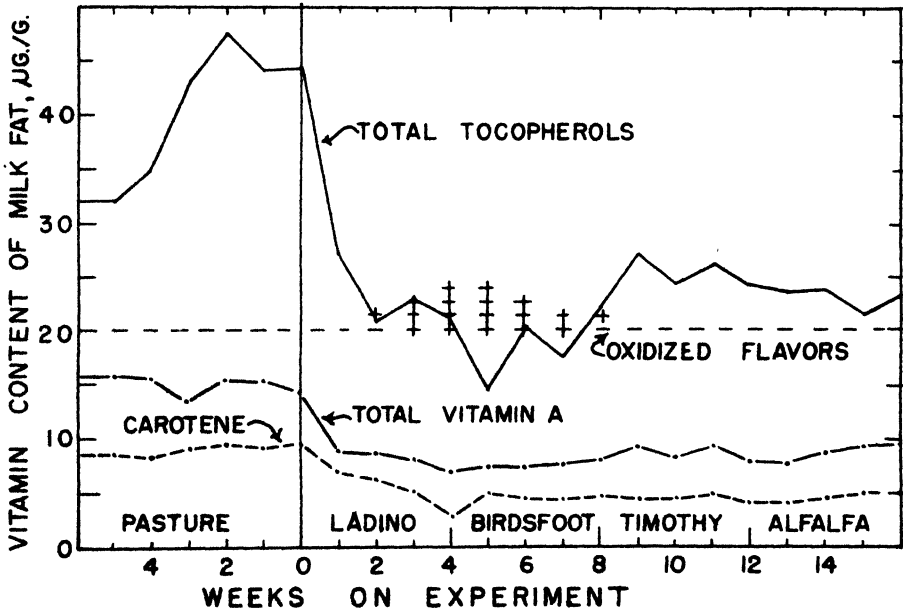


FIG. 1. Illustration of trends in total tocopherols, carotene and total vitamin A in the milk fat in the milk of a cow fed successively, birdsfoot trefoil pasture, ladino clover hay, birdsfoot trefoil hay, timothy hay and alfalfa hay. All cows showed similar responses. The minus (-) and plus (+) signs indicate absence or presence and intensity of oxidized flavors in milk.

#### SUMMARY

Two experiments are reported on the value of different hays for milk production. In the first study, 15 Holstein cows were fed six types of hay in an incomplete block design experiment involving four periods of 5 wk. each. The hays studied included early-cut timothy, late-cut timothy, second crop alfalfa cut at early and late stages of maturity, birdsfoot trefoil (*Lotus corniculatus*) and ladino clover. Measurements were made of the palatability of the hays and of their effects upon milk production, and on the carotene, vitamin A and tocopherol contents and stability of the milk.

The legume hays and early-cut timothy were approximately equal in value, but the late-cut timothy proved much less palatable and resulted in lower milk production than any of the other hays. On *ad lib.* feeding the average intake of late-cut timothy was only 35 to 44 per cent as much as of the other hays and the actual milk production was approximately 25 per cent lower.

Milk of poor keeping qualities resulted during ladino clover feeding and appeared to be correlated with a low content of tocopherol in the milk fat.

A second study with similar hays using four cows in a  $4 \times 4$  Latin square design gave data in good agreement with the first test.

#### ACKNOWLEDGMENT

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## IN VITRO STUDIES ON THE CONVERSION OF CAROTENE TO VITAMIN A IN DAIRY CALVES<sup>1</sup>

O. T. STALLCUP AND H. A. HERMAN

*Department of Dairy Husbandry, University of Missouri, Columbia*

The fact that certain carotenoids are the mother substances of vitamin A synthesized in animals is well known. However, limited knowledge exists concerning the mechanism of the conversion of carotenoids to vitamin A, particularly as it affects the site of conversion in the organism.

The early experiments of Moore (14), since amply confirmed by numerous investigators, clearly established the appearance of vitamin A in the liver of rats following oral administration of carotene. It generally has been assumed that an enzyme "carotenase," in the liver, is responsible in effecting the conversion of carotenoids to vitamin A, but direct experimental evidence of a satisfactory character has not been fully advanced to substantiate this hypothesis.

The site of conversion of carotene to vitamin A in the rat was reported to be the liver by Olecott and McCann (15) on the basis of *in vitro* experiments. Sexton (18), Rea and Drummond (17) and Ahmad (1) were unable to confirm the work of Olecott and McCann. Negative results were reported on *in vitro* experiments using shark liver by Euler and Euler (5), on cat liver by Drummond and McWalter (3), Ahmad (1) and Rea and Drummond (17). Parienti and Ralli (16) obtained one positive test out of four on dog livers, while Euler and Klussman (6) reported positive conversion of carotene to vitamin A when cow liver was incubated *in vitro* with a carotene solution. Wilson *et al.* (21) reported positive results on rabbit liver. More recently, Glover *et al.* (7), Mattson *et al.* (12), Wiese *et al.* (20), McCord and Clausen (13) and Krause and Pierce (11) have presented evidence demonstrating that the transformation of carotene to vitamin A in the rat occurs in the small intestine.

Goodwin and Gregory (8) have presented evidence that the conversion of carotene to vitamin A occurs in the intestine in the case of sheep, goats and rabbits. Klosterman *et al.* (10) suggest that carotene is converted to vitamin A during digestion and/or absorption in sheep. Swick *et al.* (19) have shown that carotene is converted to vitamin A in the intestinal wall of the pig.

Elliott (4) recently has reported an increase in the vitamin A value of the blood plasma of the intestinal wall and jugular circulation following the ingestion of carotene by dairy calves. He reported no rise in the vitamin A content of the blood plasma of the calf following intravenous injections of high-carotene cow plasma but reported that the vitamin A content of the liver increased following such injections.

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This investigation was undertaken in the hope that further information might be obtained regarding the site of conversion of carotenoids to vitamin A in dairy calves. It was decided that this conversion might be demonstrated more effectively *in vitro*. In the intact animal, it is assumed vitamin A would be removed about as quickly as formed, especially in the case of synthesis in the intestinal mucosa.

#### METHODS

The calves used in this experiment were males of the Holstein-Friesian, Guernsey and Jersey breeds dropped in the Missouri Station herd. The calves were allowed to remain with the dam for 3 to 4 days and were permitted colostrum feeding as usual. At 4 days of age they were removed from the dam and received mixed herd milk (butterfat content 3.9 to 4 per cent) until 3 to 4 wk. of age. At 2 wk. of age the calves were given free access to a "calf starter ration" low in carotene content and made up of: 400 lb. white corn, 300 lb. ground oats, 300 lb. wheat bran, 100 lb. linseed oil meal, 60 lb. non-fat dry milk solids, 20 lb. soluble blood flour and 1 lb. irradiated yeast.

Straw containing 1.87  $\gamma$  of carotene per g. (dry basis) was fed *ad libitum* as the sole source of roughage. The animals were bedded with wood shavings in individual stalls, except for a brief period each day when they were allowed to exercise in a dry lot.

The calves were slaughtered when blood plasma vitamin A values reached lowered levels ranging from 5.16 to 14.5  $\gamma$  per 100 ml. plasma. Care was exercised to prevent the animals being depleted of vitamin A to the point that deficiency symptoms occurred.

The age of slaughter and time required for depletion are shown in table 1.

TABLE 1  
*The age and the time required for depletion of vitamin A reserves of calves used in in vitro experiments*

No. of animal	Breed	Age when killed	No. of days in depletion period	Blood plasma vitamin A
		(d.)		( $\gamma$ /100 ml)
200	Guernsey	156	38	6.49
130	Holstein	127	97	7.89
489	Guernsey	135	105	5.92
528	Jersey	156	126	9.07
504	Jersey	150	120	7.77
509	Jersey	116	86	5.16
119	Holstein	121	91	14.50
495	Guernsey	153	123	11.63

The animals were stunned by a blow on the head and the left carotid artery was severed to permit hemorrhage. The small intestine was removed immediately, ligated at both ends and placed in Ringer-Locke solution<sup>2</sup> having a temperature of 38° C. Likewise, the liver was removed and placed in Ringer-Locke solution having a temperature of 38° C.

<sup>2</sup> The Ringer-Locke solution employed had the following composition: NaCl, 0.9%; KCl, 0.042%; CaCl<sub>2</sub>, 0.024%; NaHCO<sub>3</sub>, 0.05%; MgCl<sub>2</sub>, 0.02%; and glucose, 0.05%.

The contents of the small intestine were flushed out three times with warm 0.9 per cent saline solution and the washings discarded. Two 3-ft. sections then were taken from the mid-portion of the small intestine. A small amount of intestine was removed at the juncture of the two sections immediately after slaughter for analysis of carotene and vitamin A.

To one portion, 250 ml. of a colloidal carotene solution<sup>4</sup> were added and both ends ligated. This section was placed in a container of Ringer-Locke solution. The colloidal carotene solution was prepared by dissolving the carotene in acetone. The resulting solution then was mixed with water and the acetone evaporated under a vacuum.

The mixture containing the carotene was stabilized by adding 1 g. of Tween 80<sup>4</sup> to each 5 ml. of solution. The carotene concentration of the solution was varied. The levels used in each experiment are shown in table 2. Ringer-Locke solution was placed inside the other section of intestine and it too was placed in a container of Ringer-Locke solution after having been ligated. The containers into which the two sections had been deposited were placed in a glass chamber in an atmosphere of nitrogen. They were incubated for 3 hr. at 38° C. In some cases, a third section of intestine containing the carotene solution was incubated for 8 hr. at 38° C., together with the corresponding section containing no carotene, which served as a control.

After incubation, the sections of intestine were removed from the chamber, the contents flushed out three times with 0.9 per cent saline and the washings analyzed for vitamin A. The intestines were minced in a food chopper and an aliquot taken for analysis. The intestinal wall was analyzed for carotene and vitamin A by the method of Davies (2) for liver, using the Carr-Price reaction for the determination of vitamin A.

The liver was finely ground by means of an ordinary food chopper. To 150 g. of minced liver, 100 ml. of a colloidal carotene solution and 250 ml. of a buffer solution<sup>5</sup> were added. The concentration of the carotene solution used was varied. Concentrations for each experiment are given in table 2. This sample was incubated for 24 hr. at 38° C. A control sample composed of 150 g. of liver, 100 ml. of distilled water and 250 ml. of the buffer solution was incubated under the same conditions. This is essentially the same procedure outlined by Euler and Klussman (6). A sample of minced liver was analyzed for carotene and vitamin A immediately after slaughter. These samples were analyzed for carotenoid and vitamin A by the method of Davies (2).

Samples of blood plasma were incubated with a colloidal carotene solution for 24 hr. at 37° C. A control sample containing no carotene was incubated

<sup>3</sup> The crystalline carotene used in this experiment was furnished by Valley Vitamins Inc., McAllen, Texas.

<sup>4</sup> "Tween" 80 was obtained from the Atlas Powder Co., Wilmington, Delaware.

<sup>5</sup> The buffer solution was made up as follows: 39.5 ml. of 0.2 N NaOH, 50 ml. of 0.2 M acid potassium phosphate and this made up to 200 ml. with distilled H<sub>2</sub>O. The pH of this solution was 7.4.



TABLE 2  
*The in vitro conversion of carotene to vitamin A in the intestinal wall of the dairy calf*

Calf no.	Breed	Intactine (non-incubated)		Intestine incubated as control		Intestine incubated 3 hr. with carotene		Intestine incubated 8 hr. with carotene		Concentration of carotene solution
		Carotene	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	
		( $\gamma/100$ g.)	( $\gamma/100$ g.)	( $\gamma/100$ g.)	( $\gamma/100$ g.)	( $\gamma/100$ g.)	( $\gamma/100$ g.)	( $\gamma/100$ g.)	( $\gamma/100$ g.)	( $\gamma/ml.$ )
200	Guernsey	23.54	16.16			96.57	7.26			226.8
489	Guernsey			62.28 <sup>a</sup>	11.05	522.00	20.50			226.8
528	Jersey			25.94 <sup>a</sup>	5.80	848.40	41.75			226.8
504	Jersey	154.65	40.05	126.50 <sup>a</sup>	28.29	390.10	39.00	345.0	72.75	780.0
509	Jersey	103.50	17.30	46.00 <sup>b</sup>		228.3	45.60	125.8	29.10	780.0
119	Holstein	68.77	21.30	59.50 <sup>b</sup>	28.0	1,108.0	114.20	615.0	506.80	780.0
495	Guernsey	63.20	9.73	55.88 <sup>b</sup>	12.6			318.9	99.10	780.0

<sup>a</sup> Incubated 4 hr.

<sup>b</sup> Incubated 8 hr.

TABLE 3  
*The in vitro conversion of carotene to vitamin A by minced liver tissue*

Calf no.	Breed	Liver (unincubated)		Liver incubated as control		Liver incubated 24 hr. with carotene		Concentration of carotene solution
		Carotene	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	
		( $\gamma/150$ g.)	( $\gamma/150$ g.)	( $\gamma/150$ g.)	( $\gamma/150$ g.)	( $\gamma/150$ g.)	( $\gamma/150$ g.)	( $\gamma/ml.$ )
200	Guernsey	374.76	77.70			46,903.0	638.75	560.00
130	Holstein	616.50	186.60	389.50	143.52	46,962.0	641.35	560.00
489	Guernsey	623.25	211.50	550.40	232.47	50,040.0	1,145.40	560.00
528	Jersey	460.35	156.45	456.0	113.57	18,200	686.55	226.80
504	Jersey	131.25	156.30	63.35	61.48	8,920	296.70	127.60
509	Jersey	464.55	464.55	306.75	357.08	9,512.5	1,195.43	127.60
119	Holstein	302.5	266.25	268.0	232.50	8,680	416.76	127.60
495	Guernsey	478.5	483.90	246.95	190.44	8,762.5	560.62	127.60

under identical conditions. The carotene and vitamin A content of the blood plasma samples was determined by the method of Kimble (9).

#### RESULTS

The results of the incubation of the small intestine of dairy calves with a carotene solution are shown in table 2. Negative results were obtained with the intestine of calf 200 which was incubated for only 3 hr. In all other cases, there was an increase in the vitamin A content of the intestinal wall incubated with carotene above that present in the nonincubated intestine and above that present in control samples. There was no vitamin A in the material washed out of the intestine.

It is felt that the results here do not necessarily represent the rates of absorption and conversion as present in the intact animal, since the carotene is present in a different carrier. However, in spite of the difference in physiological conditions existing between *in vivo* and *in vitro* methods, it is believed that conversion of carotene to vitamin A by the intestine is clearly indicated.

In all cases, there was a conversion of carotene to vitamin A when livers from depleted calves were incubated with a carotene solution (table 3). The fact that conversion in Guernseys appeared to be equal to or in some cases higher than Jerseys and Holsteins is unexplained. It generally is believed that the conversion of carotene to vitamin A in Guernseys is less efficient in the living animal than is the case with some of the other dairy breeds. It should be emphasized that since *in vitro* experiments were carried out caution is necessary in the application of these results to the living organism.

In no case was carotene converted to vitamin A by blood plasma. This is in agreement with the results of Elliott (4) and Von Euler and Klussman (6).

#### SUMMARY

1. Data obtained from the *in vitro* incubation of small intestine of dairy calves with a colloidal carotene solution indicate that the small intestine is a site of conversion of carotene to vitamin A.

2. The incubation of minced liver tissue with a colloidal carotene solution resulted in a conversion of carotene to vitamin A.

3. Conversion of carotene to vitamin A apparently is not a function of blood plasma.

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## ASSOCIATION ANNOUNCEMENTS

*Forty-Fifth Annual Meeting*

*Cornell University*

*Ithaca, N. Y.*

*June 20, 21, 22, 1950*

### REGISTRATION AND HOUSING

Registration and housing headquarters will be in Willard Straight Hall, Cornell University, Ithaca, New York. Housing facilities will be available in University dormitories. Meals can be obtained at university cafeterias. A return card and a letter giving detailed information relative to advanced registration, housing, transportation and entertainment will be sent to members of the Association early in May.

### PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for the projection of  $3\frac{1}{4}'' \times 4''$  and  $2'' \times 2''$  slides. Those wishing other projection equipment should notify their section chairman.

### COMMITTEE AND SPECIAL MEETINGS

Those wishing rooms for section committee meetings or special meetings should contact B. L. Harrington, Stocking Hall, Cornell University, Ithaca, New York. Provision can be made for special breakfasts, luncheons or dinners by writing to S. E. Smith, Wing Hall, Cornell University, Ithaca, New York, by June 1.



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## AN ACTIVITY TEST FOR CHEDDAR AND COTTAGE CHEESE STARTERS<sup>1</sup>

B. E. HORRALL<sup>2</sup> AND P. R. ELLIKER<sup>3</sup>

*Purdue Agricultural Experiment Station, West Lafayette, Indiana*

Preliminary studies on slow acid development in the manufacture of cheddar cheese in Indiana demonstrated the necessity, in a plant starter program, for a simple, accurate test to determine the activity of starter cultures under conditions simulating those in the cheese vat. This report deals with the development of a plant test to determine activity of cheddar and cottage cheese starter cultures.

High activity in starter cultures appears to be of more importance today than in the past because of the general trend toward pasteurization of milk for cheddar cheese manufacture and consequent destruction of acid-producing organisms normally present in the raw milk.

### REVIEW OF LITERATURE

Whitehead and Cox (4) devised a method of testing the vitality of starters by simulating the cheese-making process in pint jars and testing the acidity of the whey at various intervals. This method or modifications of it have been used by a number of laboratories in this country.

Elliker and Frazier (2) determined the activity of Swiss cheese starter cultures by growing them at temperatures near their maximum in sterile reconstituted skim milk prepared from the same lot of powder. The rate of growth was determined by direct microscopic counts of living cells and also by determinations of pH and titratable acidity at hourly intervals over an 8 hr. period.

Anderson and Meanwell (1) measured the activity of cheddar cheese starters by adding a 1 per cent inoculum of starter to sterilized milk and incubating duplicate tubes for 6 hr. at 30 and/or 37° C. The acidity then was determined with 0.11 N NaOH using 1 ml. of 0.5 per cent phenolphthalein indicator per 10 ml. of the incubated milk.

Johns and Berard (3) in studying relationship between overripening and starter activity, modified the Whitehead and Cox vitality test. Fresh sterile skim milk was inoculated with 1 per cent of the culture. The samples were incubated in a water bath at 86° F. for 2 hr. and then transferred to an incubator

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<sup>1</sup> This study was made possible by a special project grant from Kraft Foods Co., Chicago, Illinois.

<sup>2</sup> Now with Kraft Foods Co., Chicago, Illinois.

<sup>3</sup> Present address: Department of Bacteriology, Oregon State College, Corvallis.

at 102° F. where they were kept for the next 4 hr. The samples were mixed carefully before sampling and were titrated for acidity every hour.

#### EXPERIMENTAL

A survey was made of activity tests for cheddar cheese starter cultures. Certain of the methods were rather time consuming and difficulty was experienced in obtaining uniform results by methods employed in the average cheese plant. Consequently an attempt was made to develop a method of determining activity of cheese starter cultures that would be simple, rapid and yet consistent. It also was believed that the method should involve a test of a culture's ability to develop at or near the cooking temperature employed in the manufacture of cheddar cheese.

*Procedure for activity test developed.* The test finally developed for activity of mother cultures and starters employs a reconstituted high grade, spray, non-fat dry milk solids for the culture medium. The use of whole milk from a selected herd as a medium for the activity test was attempted, but the results were less consistent than with the reconstituted milk. It was believed that if the same lot of milk powder should be used over a long period of time, one variable factor would be eliminated. The reconstituted milk was prepared at the rate of 10 per cent of the non-fat dry milk solids in distilled water and was heated in flasks at 15 lb. pressure for 10 min. Respective 10-ml. quantities of sterile milk were pipetted aseptically into sterile screw-top test tubes and adjusted to 37.8° C. in a water bath. Each tube was inoculated with 0.3 ml. of the starter culture to be tested and incubated at 37.8° C. for 3.5 hr. Then the entire contents of the tube, together with 5 ml. of distilled water used to rinse the tube, were titrated with 0.1 *N* NaOH to a faint pink color using phenolphthalein as an indicator. The results were recorded as per cent lactic acid. This figure was termed the activity factor of the culture.

The test outlined above, or an earlier modification which employed an incubation time of 6 hr. and a 1 per cent inoculation, has been used daily in 20 plants for a period of more than 4 yr. It has aided materially in selecting starters and cultures to be used for cheese manufacture.

*Comparative results with proposed activity test.* Figure 1 represents a comparison of the proposed test for culture activity and the Whitehead and Cox test since the latter method has been most commonly used in the past to determine activity of cheddar cheese cultures.

Many comparisons using these methods were made on mother and batch starters. One representative comparison on the mother culture, used to inoculate the batch culture subsequently employed in the cheese vat is shown in figure 1. The milling acidity shown represents the rate of acid production in the cheese by the batch starter organisms inoculated with the respective mother culture. The other two comparisons shown in figure 1 represented activity tests on batch cultures used to inoculate cheese milk.

The points plotted for the Whitehead and Cox test were the acidity of the whey from the third or last draining which was taken at the end of 6.5 hr.

These points represented the average of duplicate tests. The original method of Whitehead and Cox involves an estimation of the activity based on the difference between the last two titrations. The original method was followed, but for purpose of convenience only the last titration is included here. The milk used for the Whitehead and Cox test was whole milk from a selected herd. This test milk was pasteurized at 65.6° C. for 5 min.

The new activity test was made in duplicate and the points plotted repre-

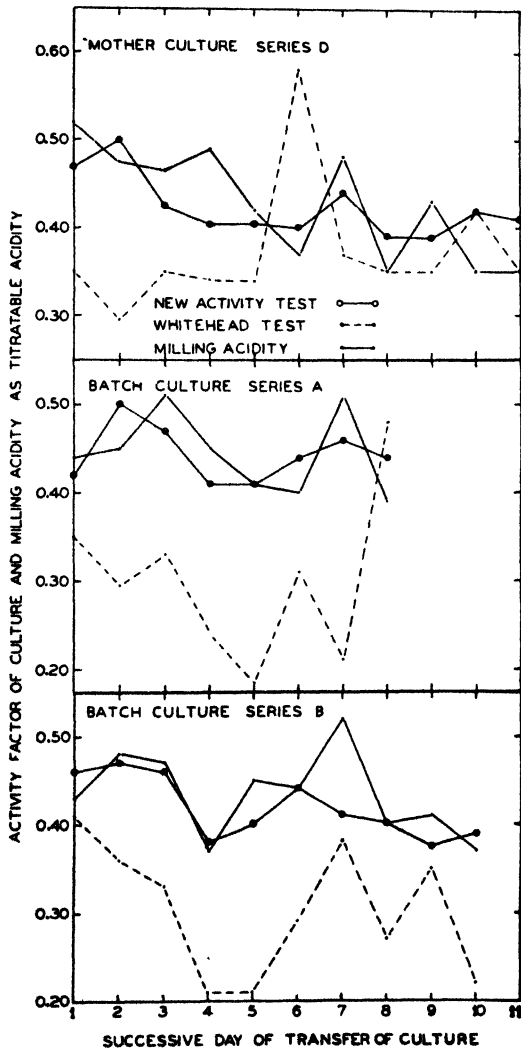


FIG. 1. Comparison of proposed activity test, Whitehead and Cox test and milling acidity of the cheese. (Milling acidity for series D necessarily represents one transfer removed from mother culture tested.)



sented an average of the results. It was found that duplicate and triplicate titrations with the Whitehead and Cox test varied considerably. One duplicate test varied 0.3 per cent and others varied as much as 0.1 per cent at the third drain, while duplicate tests with the new method varied only 0.04 per cent as a maximum. Most of the tests showed less than 0.02 per cent variation.

The batch starter (series A and B) was made with whole milk in 10-gal. cans, heated to 87.8° C. and held for 1 hr. The amount of starter used in the cheese vat was 0.7 per cent.

The curves on batch cultures series A and B suggest that the new activity test predicted more accurately the milling acidity of the cheese which is an indication of the activity of the starter. However, it is realized that the proposed test will not always predict the milling acidity of the cheese because of the

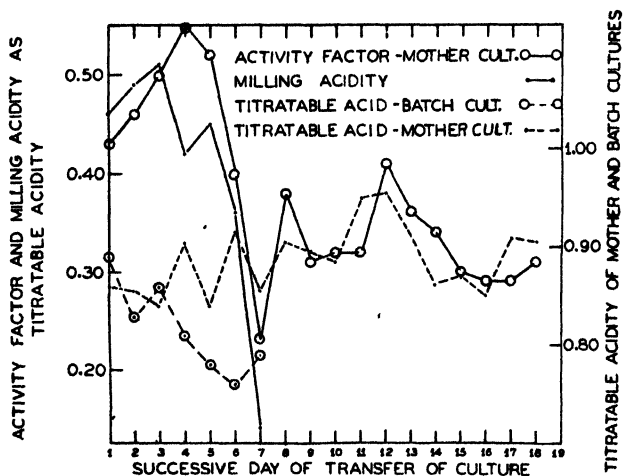


FIG. 2. Activity of one culture over a period of 18 days.

effect of such factors as variation in rate of inoculation of the vat milk, quality of the milk used, presence of bacteriophage and overheating in the vat. Starter cultures that developed sufficient acid to yield an activity factor of more than 0.35 in the activity test usually resulted in cultures that were active in the cheese vat, providing all the factors listed above were eliminated. Starters with an activity factor of 0.30 to 0.35 usually were found to develop acid more slowly in the cheese vat. A starter with an activity factor of less than 0.30 usually developed little or no acid during the cheese-manufacturing process.

*Correlation between activity factor and daily activity of successive transfers of a mother culture.* Records of activity tests made in two cheese plants on mother cultures for a period of more than 4 yr. reveal that the new test frequently indicates those cultures that are deteriorating. The results with one culture are shown in figure 2, which gives the activity factor of the mother culture, the acidity of the mother culture at the time it was transferred, the acidity

of the subsequent batch culture and milling acidity when used for cheese manufacture.

This culture at the beginning of the trial was high in activity and produced the desirable milling acid with 0.6 to 0.7 per cent inoculation in the cheese vat. It had been used in the plant about 6 wk. before any change was detected. It will be noted in figure 2 that the activity of the culture was high (0.56); then it rapidly decreased and in three transfers had an activity factor of 0.23, which is considered an inactive culture. The milling acidity of the cheese also decreased each day after the culture started to lose activity. On the last day that the culture was used in the cheese vat, the mother culture had an activity factor of 0.23 and the vat milled at 0.14 per cent acid. The propagation of the culture was continued for 11 days to revive it. On the seventh and eleventh days (as shown in figure 2), the inoculation was increased. In both instances the activity increased the following day but dropped again the next day. As shown by the activity test, the culture did not regain its earlier high activity during the 18-day period. The results also indicate that the new activity test is more sensitive in predicting inactivity than daily determination of titratable acidity of mother and batch cultures.

The new test has been used to determine the activity of multiple as well as single-strain cultures employed in cheddar and cottage cheese. It has proved a valuable aid in reducing incidence of slow acid production in the cheddar as well as cottage cheese making process.

#### SUMMARY

1. A simple, rapid test for activity of cheddar and cottage cheese cultures is described.
2. The method involves inoculation of 3 per cent of the starter culture to an autoclaved reconstituted non-fat dry milk solids medium and titration of acidity after 3.5 hr. incubation at 37.8° C.
3. The results indicate that the new activity test is reasonably accurate and consistent and is sufficiently simple to be applied readily to plant conditions. It has been employed in a number of cheddar cheese plants for more than 4 yr. and has been a valuable aid in reducing incidence of slow acid production.

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# THE USE OF RECONSTITUTED NON-FAT DRY MILK SOLIDS FOR PROPAGATING MOTHER AND BATCH STARTER CULTURES<sup>1</sup>

B. E. HORRALL<sup>2</sup> AND P. R. ELLIKER<sup>3</sup>

*Purdue Agricultural Experiment Station, West Lafayette, Indiana*

AND

GEORGE KENSLER

*Kraft Foods Co., Chicago, Illinois*

The importance of high activity in lactic starters since the introduction of pasteurization of milk for cheddar cheese generally is acknowledged. It also is known that the milk used to grow the mother and batch starters must be of the highest quality for consistently high starter activity. Whole milk usually has been used most frequently for propagating cheddar cheese starters. In some cases the mixed milk as it arrives at the plant has been used. Skim milk and whey have been employed less frequently.

Another recognized fact is that milk from individual herds varies from day to day in its ability to support growth of starter bacteria. Such variations affect the uniformity of starter activity. It was believed that if mother cultures and starters could be carried in milk of the same composition over long periods of time, more uniformity in their activity could be obtained. This report represents results of more than 4 yr. of study on the use of reconstituted spray dried, non-fat milk solids for preparation of mother cultures and starters in commercial cheese plants.

## EXPERIMENTAL

*Concentration of solids in reconstituted milk.* Preliminary studies showed that a 10 per cent concentration of spray, non-fat dry milk solids in distilled water yielded as satisfactory cultures as did 12, 15 and 18 per cent concentrations.

*Activity of mother cultures carried in reconstituted and selected herd milk.* A comparison was made of the daily activities of a mother culture carried in 10 per cent high grade, spray, non-fat dry milk solids in distilled water and in selected whole milk from an individual herd. Two hundred ml. of the reconstituted milk and whole milk were placed in respective 500-ml. erlenmeyer flasks, autoclaved at 15 lb. pressure for 10 min., cooled to 21.1° C. and inoculated with 0.7 per cent of an active culture. These milks were incubated at 21.1° C. for 15 hr. and then cooled in ice water. The activities of these cultures were tested by the method described in a preceding paper (1).

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<sup>2</sup> Now with Kraft Foods Co., Chicago, Ill.

<sup>3</sup> Present address: Department of Bacteriology, Oregon State College, Corvallis.

Results representative of numerous trials are shown in figures 1 and 2. The activity of the mother culture propagated in non-fat dry milk solids was relatively constant from day to day (the lowest activity factor was 0.40 and the highest, 0.48) while that of the whole milk varied from 0.34 to 0.51, with a slightly lower average activity factor than the reconstituted skim milk. Other trials conducted in the same manner but at different seasons also showed that

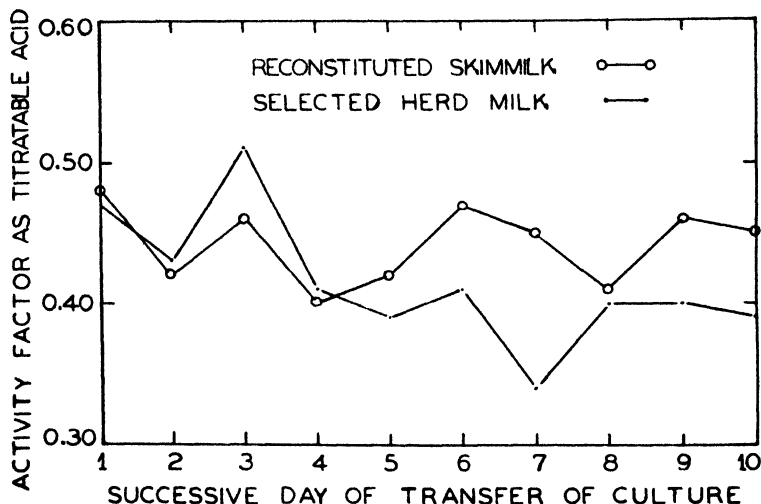


FIG. 1. Activity of mother cultures propagated in reconstituted 10 per cent spray, non-fat dry milk solids and in selected whole milk.

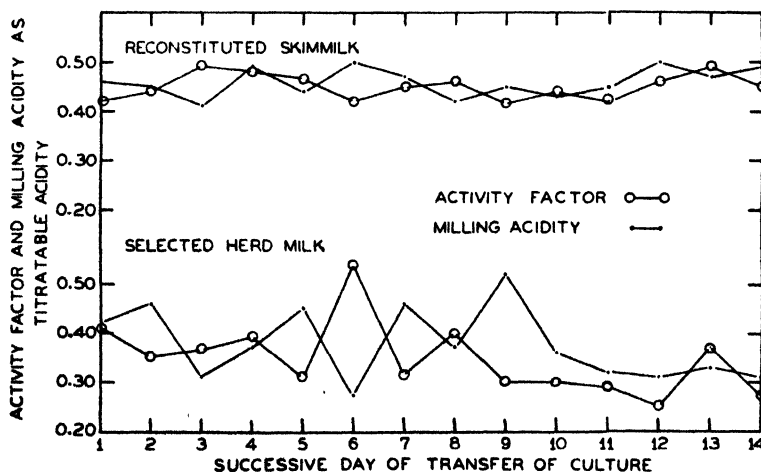


FIG. 2. Comparison of the activity of batch starters propagated in reconstituted skim milk and selected whole milk and the respective milling acidities of cheeses prepared from these starters.

the whole milk cultures usually had an average activity factor lower than that of the reconstituted, non-fat, dry milk solids cultures. In all of these trials the acidities of the whole milk cultures at the end of the daily incubation period of 21.1° C. averaged from 0.1 to 0.2 per cent lower than those of the cultures propagated in the reconstituted milk.

*Activity of batch starters prepared with reconstituted and selected whole milk.*

A study next was made of activity of batch starters prepared in reconstituted, spray, non-fat dry milk and in selected whole milk. The whole milk was from the same herd as that used in the previous experiment on mother cultures. The milks were pasteurized at 87.8° C. for 1 hr. in cans, then cooled to 21.1° C., inoculated with 0.7 per cent of an active lactic culture and incubated at this temperature for 15 hr. The rate of inoculation of the vat milk was 70 lb. of starter to 10,000 lb. of milk. The cheese was made by the precision method (2).

Results in figure 2 indicate that the starters carried in the reconstituted milk were more constant in activity than those in the whole milk. The activity factors of the starters propagated in the reconstituted milk ranged from 0.415 to 0.49, while those of the whole milk varied from 0.25 to 0.54; the respective milling acidities varied from 0.41 to 0.50 per cent for the reconstituted milk and from 0.27 to 0.52 for the whole milk starter.

Mother cultures, both multiple and single strain, have been carried continuously in the laboratory in reconstituted non-fat dry milk solids for more than 4 yr. and both mother cultures and batch starters have been propagated by this method in 20 plants during this period with satisfactory results.

*Influence of type of water used in reconstituting milk for mother and batch starters.* The large quantities of distilled water that were required for preparing the batch starter presented a problem. An experience with one plant early in the studies emphasized the importance of the type of water used for reconstituting the milk solids for starter milk. Following a preliminary failure of cultures grown in milk reconstituted with this plant water, a study was carried out on the activity of cultures grown in milks reconstituted with the plant water and with distilled water. In the trial, milks were pasteurized in four 10-gal. cans, (two cans for each reconstituted milk) at 87.8° C. for 1 hr., cooled to 21.1° C. and inoculated with 0.75 per cent culture. The cultures in milk made with the plant water (which in this case was from the city supply), after 15 hr. incubation, had activity factors of 0.23 and 0.27. The controls grown for the same period in milk reconstituted with distilled water had activity factors of 0.45 and 0.48. Since the activity factor was low on the plant water starter, 105 lb. were added in 10,000 lb. of pasteurized vat milk, whereas, only 75 lb. of the distilled water starter were used for the same quantity of milk. The acidity of the wheys 4.5 hr. after setting the milk were 0.25 per cent for the cheese made with plant water culture and 0.49 for that made with the distilled water culture. The batch starter prepared with the plant water milk produced acid normally during its incubation period at 21.1° C. and except for the activity test would have been judged as active for cheese manufacture as that made with distilled water. Following this experiment, an attempt was made to determine why use of plant

water resulted in an inactive starter. The solids content of the water was found to be 0.19 per cent, which is considered quite high. No specific toxic factor was found but occasional subsequent use for mother cultures always resulted in an inactive culture.

Water from the plant well also was available in this plant and a culture activity comparison was made of whole milk and milks reconstituted with distilled water, the city water and with plant well water. The activity factor was determined by the activity test. Tubes of each milk were inoculated and carried with daily transfers in triplicate and the activity tests repeated on 6 successive days. The city and well waters were drawn each day to prepare the reconstituted milks. The whole milk came from the same patron throughout the trial.

TABLE 1

*Activity factors of starter cultures in selected whole milk and in milks reconstituted with city water and plant well water*

Milk reconstituted w/city water		Milk reconstituted w/plant well water		Milk reconstituted w/distilled water		Whole milk	
Control <sup>a</sup>	Inoculated	Control <sup>a</sup>	Inoculated	Control <sup>a</sup>	Inoculated	Control <sup>a</sup>	Inoculated
0.19	0.22	0.18	0.47	0.18	0.46	0.20	0.43
0.19	0.21	0.18	0.44	0.18	0.45	0.21	0.44
0.19	0.22	0.19	0.48	0.18	0.47	0.20	0.40
0.19	0.24	0.18	0.48	0.185	0.49	0.22	0.41
0.18	0.21	0.18	0.46	0.18	0.47	0.21	0.39
0.18	0.21	0.18	0.47	0.18	0.47	0.20	0.35

<sup>a</sup> Control represents uninoculated, incubated milk.

The results shown in table 1 indicate that the plant well water was satisfactory for preparation of the reconstituted milk but the city water again resulted in a milk that failed to produce an active culture. The whole milk yielded active cultures for the most part, but the cultures were less uniform in activity in the whole milk than the milks reconstituted with distilled and plant well waters. The trial differentiated sharply between the milk prepared with city water and the other milks. It emphasized the need for preliminary trials in the form of culture activity tests on milks reconstituted from various waters before they can be employed for preparing reconstituted starter milks. In another plant both the city and plant well water failed to produce active cultures when used to reconstitute the milk powder. Waters from several farm wells in the same vicinity were tested and found to produce less active cultures than when distilled water was used for reconstituting the starter milks. The water from one farm well about 0.5 mi. from the plant provided an active starter culture.

*Method of testing suitability of milk powders for preparation of starter milks.*

Experience with various lots of powder has emphasized the importance of high grade, non-fat dry milk solids in preparing the starter milk and has shown that each barrel of powder must be tested to insure that the powder will produce a culture with high activity. The following method has been devised for testing the suitability of non-fat, dry milk solids for this purpose: (a) Weigh 10-g. portions of the unknown powder to be tested and of a powder whose ability to

support growth is known. Place the portions of unknown and known powder in respective flasks. (b) Measure 90 ml. of distilled water and add to each powder. Mix until free from lumps. (c) Measure exactly 10-ml. portions of the above milks into screw-cap test tubes. Prepare five tubes of the unknown and five of the known reconstituted milks. (d) Sterilize at 15 lb. pressure for 10 min. (e) Cool to 37.8° C. (Tubes not to be used immediately should be refrigerated.). (f) Inoculate one tube each of the unknown and known reconstituted milks with 0.3 ml. of an active culture. (g) Incubate in water bath at 37.8° C. for 3.5 hr. together with an uninoculated control tube of each milk. (h) At the end of the incubation period cool and determine titratable acidity on the entire contents of the tube plus 5 ml. of distilled water used to rinse the tube. Record as activity factor. (i) On the same day, inoculate one tube of the unknown milk with 0.1 ml. of the same active culture for the first serial transfer and incubate at 21.1° C. for 15 hr. Repeat for 2 successive days (three serial transfers). (j) Make activity test on each transfer at the end of incubation period.

In table 2 it will be noted that barrels 2, 18 and 34 showed higher activity factors than the known powder and the activity of the cultures did not decrease when they were serially transferred for 3 successive days in milk prepared from those barrels. Barrels 42 and 43 showed lower activity factors than the known powder. The last two barrels listed were not used for preparation of starter milk in the plant because of their low activity. It has been found that a starter of low activity will result if such powders are used for the propagation of starters.

*Method adopted for preparation of batch starter with spray, non-fat, dry milk solids.* The method developed for making the batch starter with non-fat dry solids was as follows: (a) Test both water and milk solids to be used in preparation of starter milk to determine their suitability for this purpose, using the methods outlined above. (b) For batch starter preparation measure the water into the starter can with a measuring stick or other suitable device. (c) Heat water to 26.7° C. and regulate steam so that the temperature does not rise higher than 29.4° C. (d) A detachable two bladed paddle was attached to the paddle shaft just slightly above the water line to facilitate the dissolving of the powder. The paddles were made so that they would sweep the water downward. (e) Add enough high grade, spray, non-fat, dry milk solids to provide a 10 per cent concentration. About 15 min. usually were required to dissolve the powder. (f) Remove the upper paddles, pasteurize the contents at 87.8° C. for 1 hr., and cool to 21.1° C. (g) Inoculate the reconstituted milk with an active starter culture. Slightly more culture (usually 4 to 6 oz. per 80 lb. of milk) was required than when whole milk was used. The rate of inoculation should be governed so that the acidity does not reach more than 0.95 per cent at the end of the incubation period of 15 hr. The temperature of incubation is a very important factor. It should be 21.1 to 22.2° C. for the entire period. (h) After incubation, cool to at least 7.2° C., if the starter is not to be used immediately. (i) Regulate rate of inoculation of the starter in the cheese milk according to the activity of the starter. With an active starter 50 to 60 lb. to 10,000 lb. of milk is sufficient to

TABLE 2  
Activity factors of barrel lots of spray, non-fat, dry milk solids

Date test begun	Lot no.	Bbl. no.	Unknown milk	Known milk	Activity factors of successive serial transfers in:							
					Unknown milk				Known milk			
					Start of trial	1st transfer	2nd transfer	3rd transfer	Start of trial	1st transfer	2nd transfer	3rd transfer
10/11/46	10226	2	0.18	0.19	0.45	0.45	0.44	0.45	0.40	0.41	0.41	0.43
10/11/46	KL887	18	0.19	0.19	0.43	0.45	0.44	0.44	0.40	0.41	0.41	0.43
11/ 4/46	KL887	34	0.20	0.19	0.45	0.45	0.43	0.40	0.43	0.43	0.42	0.41
11/18/46	10156	42	0.17	0.19	0.33	0.31	0.31	0.30	0.40	0.42	0.41	0.43
11/18/46	10156	43	0.12	0.19	0.34	0.36	0.31	0.34	0.40	0.42	0.41	0.43



provide a milling acidity of 0.5 to 0.6 per cent in 4.5 hr. from setting to milling. (It must be kept in mind that high cooking temperatures, poor quality milk, toxic substances and bacteriophage will hinder the acid development in the cheese regardless of the activity of the batch starter.)

#### SUMMARY

(a) Milk reconstituted from spray, non-fat, dry milk solids produced cultures and starters more constant in activity from day to day than did selected whole milk.

(b) Certain water supplies were found to be unsuitable for reconstituting milk for starter. Studies have shown that the water, if other than distilled, should be tested to determine whether or not it provides a reconstituted milk suitable for starter cultures.

(c) Spray, non-fat, dry milk solids varied in ability to provide a satisfactory culture medium for *S. lactis* starter cultures. Results showed that individual barrels of milk powder should be tested to determine their suitability for starter milk.

(d) Methods are outlined for testing suitability of water and non-fat dry milk solids for preparation of starter milk. A method also is given for preparation of reconstituted spray, non-fat, dry milk solids for batch starter.

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# THE RELATIONSHIPS AMONG CRACKED SOYBEANS FED, BARN TEMPERATURE AND THE DEGREE OF UNSATURATION OF MILK FAT<sup>1, 2</sup>

J. B. FRYE, JR.,<sup>3</sup> C. Y. CANNON,<sup>4</sup> AND E. W. BIRD<sup>5</sup>

*Iowa State College, Ames*

The results of a previous study (6) indicated a close relationship between external temperature changes and variations in the iodine value of the milk fat produced. That such a relationship may exist has been suggested by Dean and Hilditch (5) and Hilditch and Sleightholme (7). Likewise, Regan and Richardson (9) observed that when the room temperature was elevated above 80 or 85° F., an alteration occurred in the characteristics of the milk produced, which included, among other things, an increase in the unsaturated compounds of the milk fat (when the temperature was above 90° F.).

This study was conducted to secure additional information about the relationships of feeding cracked soybeans, barn temperature and the degree of unsaturation of milk fat.

## EXPERIMENTAL PROCEDURE

*Plan of experiment.* Twelve Holsteins and four Ayrshires were selected during a preliminary period (4 wk.) and divided into four similar lots. The placing of the cows into four equal groups and the allotment of the mixtures fed to each group were accomplished by random selection. Alfalfa hay was fed *ad libitum* and grain was fed at the rate of 1 lb. for each 3 lb. of milk produced. The animals were milked thrice daily.

Three experimental periods of 39, 74 and 56 days were employed. During the first experimental period groups 1 and 2 were fed rations (table 1) contain-

TABLE 1  
*Concentrate mixtures used*

Ingredients	Mixture A	Mixture B
	(lb.)	(lb.)
Cracked corn	400	400
Oats	200	200
Wheat bran	300	300
Linseed meal	125	
Cracked soybeans		125
Bonemeal	18	18
Salt	9	9

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<sup>3</sup> Present address: Louisiana State University, Baton Rouge.

<sup>4</sup> Dairy Husbandry subsection.

<sup>5</sup> Dairy Industry section.

ing linseed meal (mix A) and cracked soybeans (mix B), respectively. Then the grain mixtures were reversed and fed for a period of 74 days. During the last experimental period the animals were fed the same rations that they received during the first period.

Variations of the iodine values with chronological time during a previous study (6) indicated the advisability of carrying a control group of cows for each of the feeds being studied. In this study, groups 3 and 4 were designated as the control groups and were fed mixtures A and B, respectively, for the duration of the experiment without a change in feed.

*Cream and butter oil samples.* The cream and butter oil for chemical analyses were obtained and handled as described previously (6). The iodine value of the milk fat was determined according to the Hanus method (2). The thiocyanogen value of the fat was determined according to the method described by Jacobs (8), with the exception that the thiocyanogen solution was prepared by the method described by Arup (1). Acid numbers of the butter oil and pH determinations of the cream and butter serum provided a measure of possible changes in the quality of the cream and the fat. Acid numbers were determined by the method of Breazeale and Bird (3).

The high correlation found between mean external temperature and the iodine value of milk fat during a previous study (6) prompted the keeping of a daily record of the temperature changes in the barn in which the cows were housed during the entire experiment.

*Methods of obtaining cream samples for chemical analysis.* Data were obtained to determine whether a composite cream sample of 1 day's milking or a sample from one milking during the day should be taken for chemical analysis. Comparisons of the iodine and thiocyanogen values of the milk fat from each milking with those of the milk fat from composite sample for the same day are presented in table 2. These data indicate considerable variation among the iodine and thiocyanogen values of the fat from milking to milking. There is as much variation between milkings as exists from day to day (as determined by analysis of the composite samples). Temperature fluctuations may be one of the factors responsible for these variations.

The iodine and thiocyanogen values of the milk fat from the evening milk are in most cases higher than those for the morning and noon milkings. The iodine equivalent to the oleic acid content of the milk fat [iodine value—(iodine value—thiocyanogen value)  $\times 2$ ] does not vary in this fashion as consistently as does the iodine value.

Table 3 includes the pH determinations made on the cream and butter serum and the acid numbers of the butter oil from each milking and from a composite sample for the same day. Although there is considerable variation among the acid values of the milk fat from milking to milking, differences among the pH values of the cream and butter serum from milking to milking are virtually within the experimental error of the method, except for the data on January 23. Nevertheless, the variation encountered in the iodine, thiocyanogen and acid

TABLE 2

*Iodine and thiocyanogen values and the grams iodine equivalent to the oleic acid in 100 g. milk fat from morning, noon and evening milk and from a composite milk sample for the same day*

Date samples taken	Iodine value					Thiocyanogen value					Grams iodine/100 g. milk fat equivalent oleic acid				
	A.M.	Noon	P.M.	Av.	Comp.	A.M.	Noon	P.M.	Av.	Comp.	A.M.	Noon	P.M.	Av.	Comp.
Jan. 5	34.45	35.09	33.48	34.34	34.55	32.08	32.92	31.40	32.13	32.11	29.71	30.75	29.32	29.92	29.67
9	32.77	32.78	33.63	33.06	32.52	29.90	28.98	30.21	29.46	29.60	27.03	23.78	26.79	25.86	26.68
12	32.90	32.69	34.05	33.21	33.24	30.13	30.02	31.42	30.52	30.17	27.36	27.35	28.79	27.83	27.10
16	33.62	33.99	35.36	34.32	34.86	30.37	30.78	32.12	31.12	31.36	27.12	27.57	29.06	27.91	27.86
19	31.84	31.89	32.00	31.91	31.93	29.09	29.51	28.87	29.15	29.40	26.32	27.13	25.74	26.39	26.87
23	33.33	34.59	35.12	34.34	34.45	31.39	31.82	32.82	32.01	32.37	29.42	29.05	30.52	29.66	30.29
Av.	33.15	33.50	33.94	33.53	33.59	30.49	30.56	31.16	30.73	30.84	27.83	27.61	28.37	27.93	28.08

TABLE 3

*Acid numbers of butter oil and the pH values of cream and butter serum from morning, noon and evening milk and from a composite milk sample for the same day*

Date samples taken	Acid number of butter oil (ml. 0.1N KOH/10 g. fat)					pH of cream					pH of butter serum				
	A.M.	Noon	P.M.	Av.	Comp.	A.M.	Noon	P.M.	Av.	Comp.	A.M.	Noon	P.M.	Av.	Comp.
Jan. 5	0.358	0.536	0.702	0.532	0.420	6.85	6.84	6.87	6.85	6.87	7.05	6.99	7.09	7.04	6.99
9	0.344	0.831	0.635	0.603	0.697	6.78	6.75	6.78	6.77	6.81	6.84	6.80	6.90	6.84	7.15
12	0.447	0.669	0.447	0.521	0.559	6.83	6.83	6.84	6.83	6.79	7.12	7.10	7.09	7.10	6.94
16	0.466	0.448	0.511	0.475	0.349	6.83	6.80	6.81	6.81	6.81	6.94	6.89	6.93	6.92	6.87
19	0.332	0.529	0.412	0.434	0.435	6.84	6.89	6.87	6.86	6.87	6.96	7.04	7.04	7.01	6.92
23	0.340	0.560	0.609	0.503	0.520	7.29	7.07	6.78	7.04	6.85	7.00	6.76	6.80	6.85	6.86
29	0.269	0.215	0.233	0.239	0.296	6.73	6.67	6.71	6.70	6.71	6.92	6.99	6.92	6.94	6.86
Av.	0.365	0.541	0.507	0.471	0.468	6.88	6.84	6.81	6.84	6.82	6.98	6.94	6.98	6.96	6.94

values of the milk fat from milking to milking indicate that daily composite cream samples should be taken.

As a check on the deterioration of the cream from the time it was separated until churned, pH determinations on the cream and butter serum were made periodically. These data (not shown) indicate that in-so-far as pH values and titratable acidities are criteria, there was no change in the cream or butter during the interval between churning and (later) rendering to furnish fat for the iodine and thiocyanogen values.

*Chemical constants of milk fat—effect of temperature.* The iodine and thio-

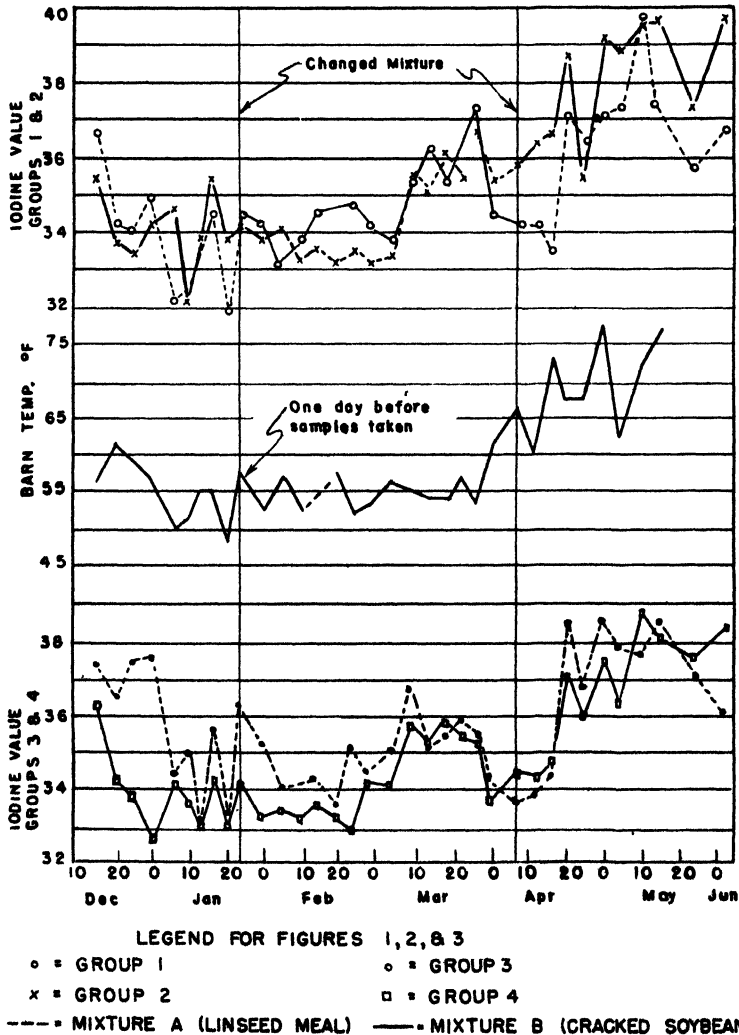


Fig. 1. Variation in iodine value and barn temperature with chronological time.

cyanogen values were employed as indices of the effect of feeds on changing milk fat composition. Previous work (6) indicated that temperature changes may play an important role in the variations of the iodine value of milk fat. A closer relationship existed between the iodine value of milk fat and the mean external temperature recorded 1 day before the samples were taken than that recorded on the same day or 2 days before. This relationship also was evident in this study.

Highly significant correlations were found between the iodine values of the milk fat and the mean barn temperatures (table 4) that were recorded the same day, 1 day before, and 2 days before the samples were taken. As the temperature increased, the iodine values tended to increase and vice versa (fig. 1). However, a closer correlation existed between the temperature recorded 1 day

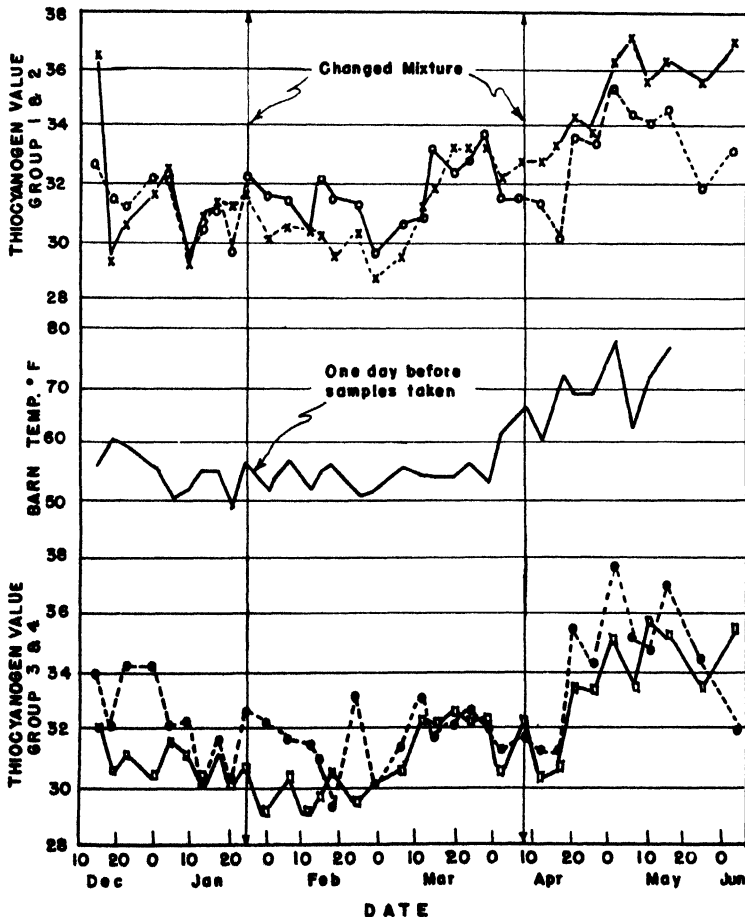


FIG. 2. Variation in thiocyanigen value and temperature with chronological time. (See fig. 1 for legends.)

before the samples were taken and the iodine value than that recorded on the same day or 2 days before. A closer correlation also existed between the temperature recorded the same day and the iodine value than that recorded 2 days before the samples were taken. The trends of the thiocyanogen values and their relationship to temperature changes (fig. 2) were similar in most respects to those of the iodine values. Generally, what was said about iodine values and the factors that influence them also may apply to the thiocyanogen values.

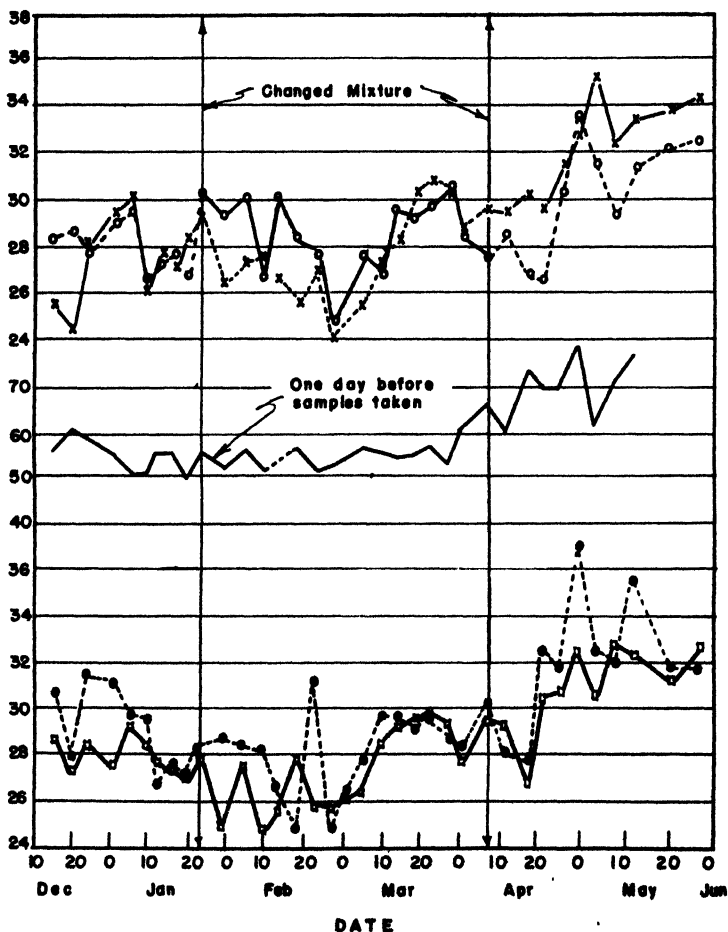


FIG. 3. Variation in oleic acid content (expressed as g. iodine/100 g. milk fat equivalent to oleic acid) and temperature with chronological time. (See fig. 1 for legends.)

The trends of the iodine, thiocyanogen and oleic acid (expressed as grams iodine per 100 g. milk fat equivalent to oleic acid) curves (fig. 1, 2, 3) are almost identical for each group. This is particularly true of the thiocyanogen curves of groups 3 and 4 (controls) (fig. 2). Since iodine and thiocyanogen are considered to add quantitatively to the single ethylenic linkage of oleic acid and

since there is more oleic acid than any of the other unsaturated fatty acids in milk fat, one would expect variations in the oleic acid content of milk fat to result in similar variations of the iodine and the thiocyanogen values. A significant correlation coefficient was found between the temperature recorded 1 day before the samples were taken and the oleic acid content of the milk fat (table 4).

TABLE 4

*Correlation coefficients between mean barn temperature recorded at various intervals and certain milk fat constants*

Temperature <sup>a</sup>	Outcome group	Iodine value	Correlation coefficient		
			Thiocyanogen value	Oleic acid <sup>b</sup>	Linoleic acid <sup>c</sup>
Same d.	1	0.3526*	0.4488**	0.2942	-0.1101
1 d. before	1	0.5103**	0.5593*	0.4022*	0.0119
2 d. before	1	0.3046	0.3653*	0.3058	-0.0468
Same d.	2	0.6076**	0.5199**	0.3879*	0.1430
1 d. before	2	0.7628**	0.6261**	0.5725**	0.0436
2 d. before	2	0.5522**	0.4945**	0.1709**	0.0580
Same d.	3	0.4573**	0.5408**	0.5290**	-0.3912*
1 d. before	3	0.5358**	0.6139**	0.6006**	-0.4030*
2 d. before	3	0.2756	0.3220	0.3307	-0.2410
Same d.	4	0.5306**	0.8939**	0.4391*	-0.0357
1 d. before	4	0.6843**	0.7128**	0.6858**	-0.2543
2 d. before	4	0.4571**	0.4858**	0.5027*	-0.2656

<sup>a</sup> Temperature recorded at various intervals with relationship to the time the cream samples were taken.

<sup>b</sup> Grams iodine calculated as equivalent to oleic acid in 100 g. milk fat.

<sup>c</sup> Grams iodine calculated as equivalent to linoleic acid in 100 g. milk fat.

\* Significant at 5% level.

\*\* Significant at 1% level.

These data indicate that the changes in iodine value were largely dependent on the changes in the oleic acid content of the milk fat.

The high correlation between temperature changes and changes in the chemical composition of milk fat support the statement made previously in this paper that temperature fluctuations may be one of the factors responsible for daily variations of fat constants, even though the cows were fed the same feed.

*Effect of feed.* Results obtained in a previous study (6) indicated that the maximum effect of feed on the changes in milk fat composition, as measured by the iodine value, may be reached in approximately 15 days after a change in feed. However, the data obtained in this experiment (fig. 1) indicate that the time necessary for the feeds to produce their maximum effect on milk fat composition is not clear, except after the first change of feeds.

Following the change of rations on December 15, the differences between the iodine value curves of groups 3 and 4 (controls) became greater than did those between the curves of groups 1 and 2 (rations alternated). These differences decreased in magnitude and seemed to stabilize themselves fairly well in approximately 20 days. Concurrent with this stabilization period was a sharp drop in temperature and likewise a downward trend of the iodine value curves of all groups. In support of these findings, Dean and Hilditch (5) observed that



changes in iodine values were completed within 2 or 3 wk. after cows had gone on pasture in the spring. During the period December 15 to January 23, temperature and iodine value fluctuations were erratic. After this it was not possible to determine when the full effect of the feeds on milk fat composition had been reached.

The differences between the curves of groups 3 and 4 were smaller and more consistent throughout the experiment than were those between the curves of groups 1 and 2. This might be interpreted as indicating that the cows in groups 3 and 4 adjusted to the feeds so that the magnitude of the iodine values was about the same regardless of the feed. The recurrent crossing and the inconsistent differences of the curves of groups 1 and 2 as compared with those of groups 3 and 4 might indicate that the fat metabolism of the cows was being disturbed by the changes in feed.

*Iodine value and hardness of butterfat.* Coulter and Hill (4) found that the hardness of butter made under uniform conditions is closely dependent upon the hardness of butterfat. They also obtained a highly significant correlation between the hardness of butterfat and the iodine number.

In this study the iodine values of the milk fat from the animals (group 3) fed the linseed meal ration generally were higher than were those (group 4) fed the soybean ration. Moreover, an analysis of variance revealed that the differences, which were one unit or less in most cases, were significant ( $F = -4.19$ ). However, it is doubtful whether a difference of one unit in iodine value would greatly affect the commercial processing of butter unless the iodine value is approximately 33 units (10). There was no significant difference between the iodine value of the milk fat from groups 1 and 2. Averages (35.1 and 35.3 for groups 1 and 2, respectively) for the entire experiment indicate that the differences between the iodine values were balanced during the experiment. However, the authors wish to point out that the differences at certain periods probably were great enough to produce varying effects on the quality of butter. For example, the differences in the iodine values of groups 1 and 2 from April 7 to 15, and from April 25 to June 1 (fig. 1) indicate that one could expect a difference in the body of butter produced by cows fed cracked soybeans or linseed meal at the same rates as were fed in this experiment. Although the iodine values of milk fats from cows fed either of these two feeds may be practically the same (especially over a long feeding period) at certain times, they also may be quite different at various temperature levels or at different stages of lactation or gestation. The feeding program used for the cows of groups 1 and 2 would not normally be followed by a dairy farmer, whereas that used for groups 3 and 4 would be more generally practiced.

#### SUMMARY

By randomization four similar lots of four cows each were subdivided into four outcome groups and fed similar rations, except for the protein supplements (linseed meal and cracked soybeans). Two groups of cows were fed rations by the double reversal method, while a control group was fed each of the experi-

mental rations continuously throughout the experiment. Iodine, thiocyanogen and acid values of the milk fat and the pH of the cream and butter serum were determined. Barn temperatures were recorded daily.

In studying changes in fat composition, it seems advisable to utilize fat samples representative of one full day's milk yield, rather than samples obtained from individual milkings.

Highly significant correlations existed between the mean barn temperature (recorded the same day, 1 day before, and 2 days before the cream samples were taken) and the iodine and thiocyanogen values of the milk fat. The temperature recorded 1 day before the samples were taken had a closer correlation to changes in fat composition than that recorded the same day or 2 days before they were taken. The authors wish to point out that the correlation between the iodine and thiocyanogen values of the milk fat and temperature existed with cows which were from 1 to 2.5 mo. along in lactation when the experiment started (late November). It might be that had the animals freshened during November, February or April a different picture would have resulted (relative to apparent temperature effects) because of the lactation cycle.

The maximum effect of changing feeds on milk fat composition, as measured by the iodine and thiocyanogen values, appeared to have been reached in approximately 20 days after the first change of feeds. Apparently such factors as temperature changes and progress of the lactation and gestation periods interfered with fat metabolism so that it was impossible to determine when the full effect of the feeds had been reached following succeeding changes. Unless certain uncontrolled factors complicate the picture it appears from this study and a previous one (6) that 15 to 20 days may be required for a feed to exert its full effect on milk fat composition. This is in agreement with the work of Dean and Hilditch (5).

When the cows were fed either linseed meal or cracked soybeans as 11.1 per cent of the concentrate mixture over a long feeding period, they appeared to adjust themselves to the rations so that the iodine values of their milk fat were of about the same magnitude and their differences fairly consistent. On the other hand, when these feeds were fed for short feeding periods and then changed, the fat metabolism of the cows seemed to be disturbed so that the differences between the iodine values of the milk fat were rather inconsistent.

The changes in iodine values were largely dependent on the changes in the oleic acid content of the milk fat.

Non-significant differences were found between the iodine values of the milk fat from cows alternated from the linseed meal ration to the soybean ration and vice versa. However, the differences at certain periods were probably great enough to produce different effects on the quality of butter. A significant difference was found between the iodine values of the milk fats from cows fed linseed meal and cracked soybeans continuously at the rate of 11.1 per cent of the concentrate mixture. The iodine values of the milk fat from cows fed the linseed meal ration generally were higher throughout the experiment than were those of the milk fat from cows fed the cracked soybean ration. Although the

iodine values of the milk fat from cows fed either of these two concentrates may be practically the same (especially over a long feeding period) at certain times, they also may be quite different at various temperature levels or at different stages of lactation or gestation.

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# THE RELATION BETWEEN THE DEGREE OF SOLIDIFICATION OF FAT IN CREAM AND ITS CHURNING TIME. II. THE PHYSICAL DISTRIBUTION OF THE LIQUID-SOLID PHASES WITHIN THE GLOBULE

J. ROBERT BRUNNER

*Michigan Agricultural Experiment Station<sup>1</sup>*

AND

E. L. JACK

*Division of Dairy Industry, University of California*

The influence of variations in churning temperature of cream upon its churning time is common knowledge. As the temperature of cream is lowered, within the churning limits, the churning time is increased. It is believed that some of the variations in churning result from changes in the degree of solidification of the fat. This supposition is well supported by observation. Undoubtedly, lowering the temperature of the cream results in greater crystallization of the fat. The fact that satisfactory churning occurs when the temperature of the cream is within rather restricted limits suggests that, at churning temperatures, the degree of solidification in the fat is quite specific.

The studies of van Dam and Burgers (4), van Dam (3), Rishoi and Sharp (9), Jack (6), Coulter and Combs (2) and others offer evidence that milk fat exists in a state of partial solidification, both in the cream at the churning temperature and in the churned butter. The extent of fat solidification is somewhat controllable by process temperature manipulation. Haglund *et al.* (5), Richardson and Abbott (8), Coulter and Combs (2), Wilster *et al.* (11) and others have emphasized that such measures often are necessary to produce a butter with desirable body and texture when made from milk fats that are abnormally hard or soft.

The purpose of this study was to correlate the degree of solidification in the fat with the churning time of the cream and to determine to what extent processing procedures affect the solidification of fat in cream prepared for churning.

## EXPERIMENTAL METHODS

To determine the extent that processing procedures affect the degree of solidification in globular fat, the following experimental procedure was followed: One hundred pounds of freshly-separated cream testing 33 per cent fat was pasteurized at 66° C. for 30 min. One-third of this cream, designated as lot A, was cooled and held at 0° C. for 18 hr. prior to churning. A second portion of the cream, lot B, was cooled and held at 10° C. for 18 hr. The remaining portion of the cream, lot C, was held at 60° C. while 5-lb. quantities were removed, cooled in ice-water to the churning temperature and immediately churned in a tempera-

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ture-controlled, motor-driven Daisy churn of an agitator type. Likewise, after cream lots A and B had been held for the designated 18-hr. holding period, 5-lb. portions were removed, adjusted to the churning temperature and immediately churned. A calorimetric technique, suitable for measuring the percentage of solid fat in cream at the churning temperature, as used previously by Jack and Brunner (7), was employed to determine the degree of solidification. Churning temperatures were selected to cover the churning range.

#### RESULTS

When churning times of cream are plotted as ordinates against percentage of solidified fat therein (degree of solidification) as abscissa, curves are obtained which, in the desirable churning range of 35 to 55 min., are logarithmic (figure 1).

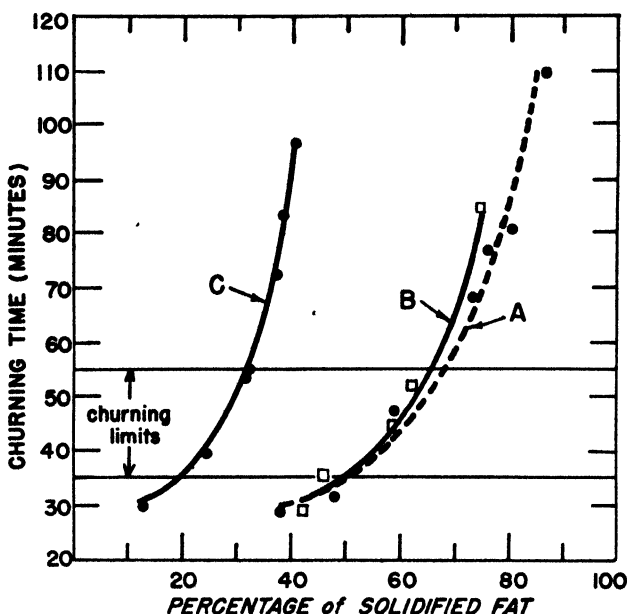


FIG. 1. The relation between the degree of solidification of milkfat at the churning temperature and the churning time of the cream (33.0% fat). Curve A—Cream held at 0° C. for 18 hr. and warmed to the churning temperature. Curve B—Cream held at 10° C. for 18 hr. and warmed to the churning temperature. Curve C—Cream pasteurized at 66° C. and cooled to the churning temperature.

Below these time limits, churnings are incomplete and accompanied by excessive fat losses in the buttermilk, while those above proceed with difficulty, if at all. Within the desirable churning range, creams from lot C contained between 20 and 31 per cent solidified fat, while creams from lots A and B contained between 48 and 68 per cent of the fat in the solid state. Cream from lot A contained slightly more crystallized fat than cream from lot B, which would be expected, since more fat should be solidified at 0° C. than at 10° C. The more fat that is

in a solid state, the greater is the time required for churning; however, within the three lots studied, the churning time depends more upon the cooling procedure used than on the percentage of solidified fat. For example, as shown in figure 1, cream from lot A, held at 0° C. for 18 hr. prior to churning, and lot C, churned immediately after cooling, containing approximately 40 per cent solidified fat churned in 30 and nearly 100 min., respectively.

The fact that a wide variation exists between the percentage of solid fat in churnings from lots A and B and lot C indicates that the degree of solidification

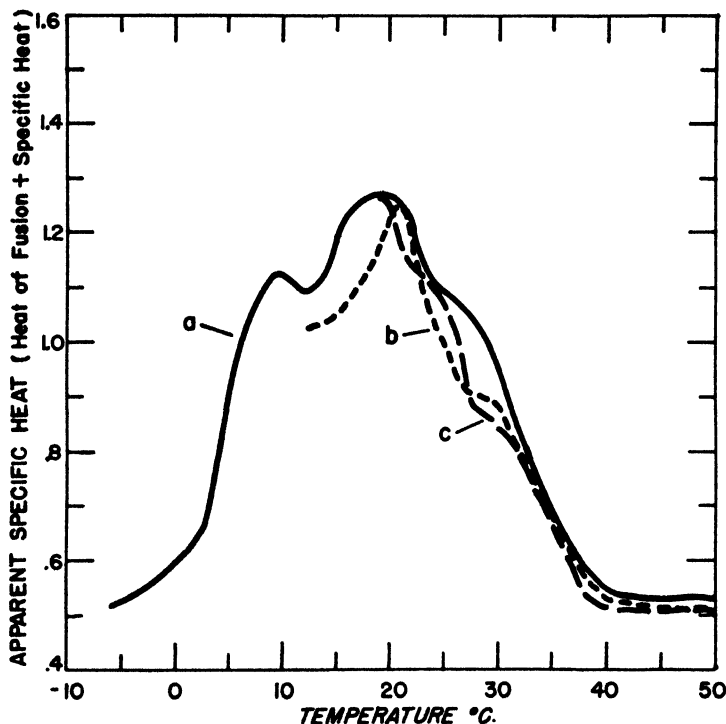


FIG. 2. Apparent specific heat values over the temperature range studied of fat in cream held at 0° C. for 18 hr. and warmed to the churning temperature. Curve a—Control, cream held at 0° C. for 18 hr. Curve b—Cream churned at 11.10° C. Curve c—Cream churned at 13.90° C.

occurring at the time of churning is not nearly as significant in the time required for churning as is the probable position of the solidified fat in the globule. Some insight as to the general location of this solidified globular fat might be obtained (a) by applying the principle of the second law of thermodynamics which states, essentially, that heat is conducted from an area of high heat level to an area of low heat level until a state of temperature equilibrium is reached and (b) from an inspection of the apparent specific heat curves of the fats involved, which, together with the work of van Dam (3) and Rishoi and Sharp (10), show that fat

exhibits a considerable time lag in attaining thermal equilibrium throughout the fat globule.

The globular fat in cream as prepared from lot A presumably is in a state of nearly complete solidification. When portions of this cream are warmed to the churning temperature, heat is transmitted to the continuous serum phase from which it is transferred to the fat phase. Due to the poor thermal conducting properties of fat, the heat transfer process proceeds inward through the fat at a relatively slow rate. This lag in attaining equilibrium probably results in the creation of a layer of partially liquid or softened fat on the outer surfaces of the fat globule. To support this assumption it is necessary to demonstrate that the

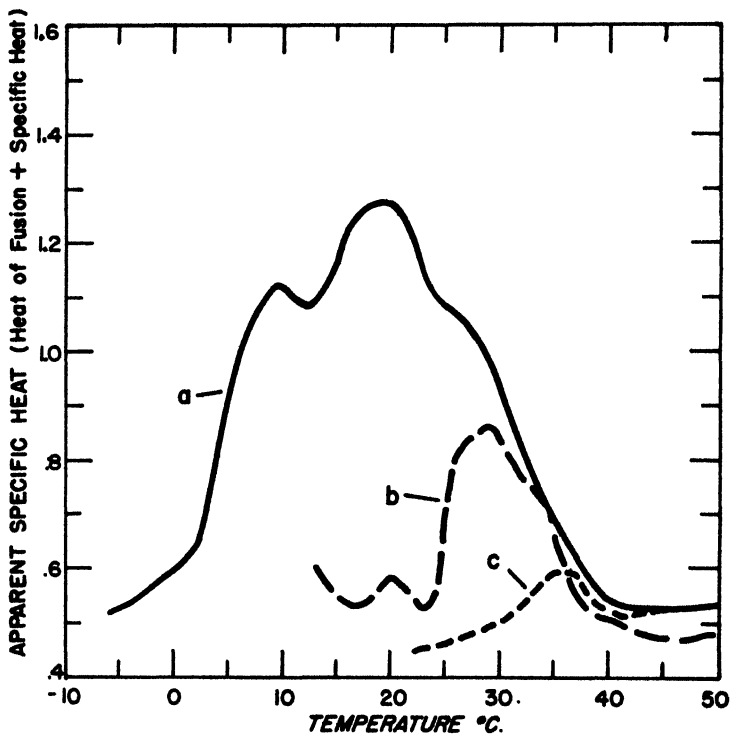


FIG. 3. Apparent specific heat values over the temperature range studied of fat in cream pasteurized at 66° C. and immediately cooled to the churning temperature. Curve a—Control, cream held at 0° C. for 18 hr. Curve b—Cream churned at 10.60° C. Curve c—Cream churned at 19.10° C.

remaining portion of solidified fat, supposedly located in the interior of the fat globule, has not been liquified in the warming process.

In figure 2, the apparent specific heat curves of fat from churnings b and c, cream processed as in lot A, are compared with a similar curve of fat in a state of nearly complete solidification (curve a). The partial curves b and c coincide fairly well, in the range covered, with that of the control, curve a. Since ap-

parent specific heat measurements are made by determining the amount of heat necessary to change the temperature of the material, this indicates that the fat being liquefied from the churning temperature upward to a state of complete liquefaction is fat in the original state of equilibrium. Therefore, the heat applied to the cream system in raising the temperature from 0° C. to the churning temperature probably was manifested in melting or softening outer layers of the globular fat, rather than being distributed evenly throughout the globule, since the residual solid fat is still in the state of equilibrium existing at 0° C.

Similar reasoning can be applied to creams prepared for churning as in lot C. When cooling cream from 60° C. to the churning temperature, heat is transferred from the serum to the cooling medium, then by a somewhat slower process from the fat globules to the serum. By this process, there probably is formed a layer of solid or liquid-solid fat surrounding the fat globules, leaving a large portion of the interior fat in a liquid state. In figure 3, the apparent specific heat curve *a*, like curve *a* in figure 2, is representative of globular fat in a state of nearly complete solidification, while curves *b* and *c* represent churning samples. At the time churning was started, immediately after the cream was cooled, and at the beginning of apparent specific heat determinations, very little of the fat had been solidified. However, after a period of 20 to 30 min. in the calorimeter, some crystallization of the fat occurred, as indicated by the maxima in curves *b* and *c*. As assumed in the preceding case, this change in physical state occurred principally in the fat globule surface layers.

The conjecture is made that the physical state of the globular fat on the surface layers is somewhat similar in creams that churn within desirable churning limits, regardless of the procedure used in preparing the creams for churning. The fat on the surface of the globule is neither totally liquid nor totally solid but in a soft semi-solid, sticky condition conducive to fat globule agglomeration. The fact that more solidified fat is formed in one method of processing than in another has little influence upon the churning time, but is manifested more significantly in the completeness of churning, as well as in the body and texture of the final butter.

Richardson and Abbott (8) have indicated that cream cooled to churning temperature immediately after pasteurization should be churned at a lower temperature than cream that has been warmed to churning temperature. The data given in figure 4 show that cream samples prepared by cooling from the pasteurization temperature (lot C) required somewhat higher temperatures to effect satisfactory churnings in the same period of time than did creams warmed to the churning temperature (lots A and B). By cooling the cream with ice-water, it is possible that a layer of solidified fat was formed on the fat globule surface which interfered with fat coalescence beyond the effect of agitation, resulting in retarded churnings. This supposition is in harmony with the reasoning advanced in this report.

In attempting to analyze the results obtained from a churning study of this nature the possibility of alteration in crystalline form should not be neglected as a means of explaining some of the variations in the churning behavior of



cream. Clarkson and Malkin (1), employing X-ray diffraction technique and melting curves, were able to demonstrate in pure triglycerides three distinct polymorphic crystal formations depending upon the rate of solidification. Slow cooling results in the formation of the most stable form designated by them as beta and having the highest melting point. More rapid cooling results in the production of an intermediate alpha form, while extremely rapid chilling will produce the unstable gamma or "glass" form. Upon aging or the application of heat, the unstable monotropic gamma and alpha forms will transform irreversibly into the more stable beta form.

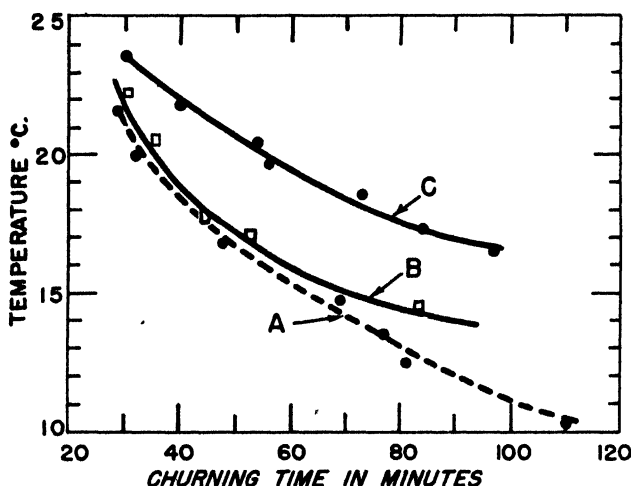


FIG. 4. The relation between the churning time of cream and the churning temperature. Curve A—Cream held at 0° C. for 18 hr. and warmed to the churning temperature. Curve B—Cream held at 10° C. for 18 hr. and warmed to the churning temperature. Curve C—Cream pasteurized at 66° C. and cooled to the churning temperature.

X-ray diffraction technique and melting curve studies, representing the best methods available for observing polymorphic transitions in triglycerides, when used to study the pure and simple triglycerides give only qualitative data; indeed, the difficulties encountered in trying to observe polymorphic transitions in a naturally occurring, mixed glyceride such as milk fat in the globular state would present a technical problem of some magnitude. Hence, certain facts obtainable from a study of the more simple glycerides are used to help explain phenomena occurring in the more complex natural fats.

The temperature history of cream samples churned from lots A and B indicate that for all practical purposes the fat is in a state of stable equilibrium (Malkin's beta crystals) at the end of the 18-hr. tempering period (Rishoi and Sharp, 10). Upon warming the cream, the added heat is expended in melting a portion of the solidified fat and raising the over-all temperature to the desired churning temperature. The possibility of reverse polymorphic transition is precluded, since the gamma alpha beta transitions are monotropic. On the other hand, when

cream samples from lot C are prepared for churning by relatively quick cooling from 60° C., the opportunity exists for the formation of the intermediate alpha and possibly the unstable "glass" types of crystallization. It is the authors' postulation, however, that if these polymorphic forms are crystallized their existence at the churning temperature would be relatively short-lived, since the transition of the unstable forms to the stable beta form is the normal transition pattern. Therefore, it is felt that the churning data obtained in this study are influenced primarily by the occurrence and distribution of solid and liquid fat in the globule as deduced from the specific heat curves shown herein. However, the possible role played by different polymorphic forms of crystalline milk fat should not be disregarded entirely, even though the data presented are not designed to demonstrate polymorphic transitions.

#### SUMMARY

A calorimetric technique was used to determine the percentage of crystallized milk fat (degree of solidification) present in creams prepared for churning by the following methods:

Lot A. Cream was cooled and held at 0° C. for 18 hr. prior to churning at selected temperatures.

Lot B. Cream was cooled and held at 10° C. for 18 hr. prior to churning at selected temperatures.

Lot C. Cream was cooled in ice-water from 60° C. to the desired temperature and immediately churned.

The degree of solidification in creams from lots A and B, churning within 35 to 55 min., ranged between 48 and 68 per cent solidified fat. Cream portions from lot C that churned within the same time range contained between 20 and 31 per cent solidified fat.

Within any one lot of cream, the more solidified fat that is present at the churning temperature, the longer is the time required for churning.

The experimental results indicate that the churning time is not entirely dependent upon the actual degree of solidification in the fat, but, to a greater extent, upon the distribution of the liquid and solid fat phases on the globular fat surfaces.

Polymorphic transition of the crystallized fat has been considered as a possible explanation for the churning results obtained.

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# ELECTROPHORESIS OF MILK PROTEINS. I. SOME COMPARISONS OF SALT-ACID AND SALT-LYOPHILIZED WHEY FRACTIONS<sup>1, 2</sup>

WILLIAM G. STANLEY, A. C. ANDREWS AND C. H. WHITNAH

*Department of Chemistry, Kansas State College, Manhattan*

There are several known methods used for the isolation of whey proteins from milk. Harland and Ashworth (3) have outlined a salt-acid method which is perhaps one of the simplest and easiest. Their procedure involves the precipitation of casein by saturation of skim milk with NaCl followed by precipitation of the whey proteins upon addition of HCl to the resulting filtrate. Harland and Ashworth claimed that whey proteins prepared by this method were "relatively undenatured." However, it is possible that addition of HCl to the non-casein filtrate might change the characteristics of the original whey proteins. Such changes in these protein fractions have been suggested by Whitnah *et al.* (13) in their studies of the surface tension of milk. Differences between the electrophoretic patterns for Harland and Ashworth whey proteins and for those obtained from whey proteins isolated by other methods should show possible changes in the whey proteins due to the effect of the precipitating reagents. The usual procedure employed in the preparation of whey proteins for electrophoretic studies involves the precipitation of casein from skim milk by acid (Smith, 10) or rennet (Deutsch, 2), followed by the concentration of the casein-free proteins by lyophilization.<sup>3</sup>

Harland and Ashworth proteins and whey proteins prepared by a modified Harland and Ashworth procedure were used in the present study. In the latter method the whey proteins were concentrated by lyophilization of the non-casein filtrate, rather than by acid precipitation. Any differences between these two patterns should indicate an effect of acid precipitation on the whey proteins. Comparison of the electrophoretic components of each of these preparations with corresponding components in the preparations of Smith and Deutsch might indicate possible effects due to the salting out of the casein.

Harland and Ashworth concluded that their "non-protein filtrate" was completely free from whey proteins and that the remaining nitrogenous substances were not protein in character. In the salt-lyophilized method of the present study this filtrate was still present in the sample. Electrophoresis should prove the presence or absence of any whey proteins not precipitated or of any non-dialyzable, non-protein nitrogenous substances in this non-protein filtrate.

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<sup>1</sup> Contribution no. 399. Department of Chemistry, Kansas Agricultural Experiment Station, Manhattan.

<sup>2</sup> This paper contains part of the material presented by William G. Stanley in a thesis for the Master of Science Degree at Kansas State College. 1950.

<sup>3</sup> In this paper the word "lyophilize" indicates the removal by sublimation in a vacuum of some water from a frozen solution.

## PROCEDURE

Pooled whole raw milk for this work was obtained from the Kansas State College dairy. The cream was separated from the skim milk with a Sharples super centrifuge. The skim milk was saturated with NaCl at 40° C. and allowed to stand overnight at this temperature. The casein was filtered off and discarded. The non-casein filtrate was divided into two parts, A and B (fig. 1). Part A was

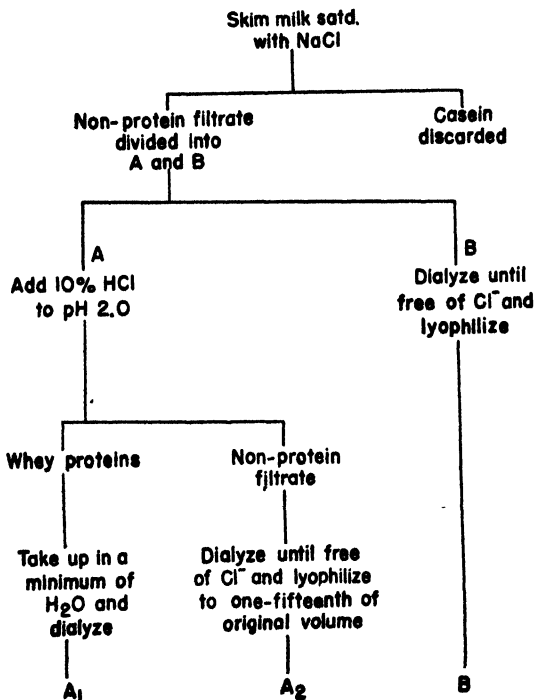


FIG. 1. Flow sheet for preparation of fractions of milk proteins.

adjusted to pH 2.0 by addition of 10 per cent HCl with rapid stirring and allowed to stand overnight at room temperature. This standing both at 40° C. and at room temperature was a definite part of the Harland and Ashworth procedure. The precipitated whey proteins were collected on a filter paper and transferred to a cellophane dialysis sack with a minimum of distilled water. Whey proteins prepared by this method were similar to the Harland and Ashworth proteins and designated as A<sub>1</sub> in figure 1. The non-protein filtrate from A was dialyzed against distilled water at 4° C. until free of chloride ion. The non-protein filtrate was then lyophilized to one-fifteenth of its original volume and designated as A<sub>2</sub>. Part B was dialyzed against water at 4° C. until free of chloride ion and then lyophilized. This fraction was designated as B.

Fractions A<sub>1</sub> and B were diluted with a veronal-citrate buffer of 0.088 ionic strength and pH 7.6 until the protein concentration was approximately 0.85 per

cent and dialyzed in 2-l. bottles at 4° C. for 72 hr. against three changes of buffer solution. Sample A<sub>2</sub> was dialyzed without dilution in the same manner. The final buffer solutions, against which the samples were dialyzed, were used as the solvents in the electrophoresis cells to form the boundaries for migration. The veronal-citrate buffer employed was prepared by bringing a solution of 206.18 g. of sodium barbital and 45 g. of sodium citrate dihydrate to pH 7.6 by the addition of citric acid and diluting to 20 l. with distilled water.

Electrophoresis was carried out for 7,680 sec. at 0.5° C. using a modified Tiselius apparatus manufactured by the Klett Manufacturing Co., New York City (1, 4, 5, 6). A constant current of 20 ma. for the electrophoresis was supplied by the current regulator as designed by McColloch (7). Patterns were recorded on Kodak Metallographic plates using the Schlieren scanning method (5).

To facilitate the measurement of peak areas, the patterns were enlarged approximately four times by projecting them on a sheet of paper. The enlarged images were traced on the paper and component areas measured with a planimeter. In determining the area due to a given component, a more or less arbitrary division of a pattern had to be made due to the overlapping of the peaks. Following the procedure of Tiselius and Kabat (12), an ordinate was drawn from the lowest point between two adjacent peaks, while use of the method of Svedberg and Pedersen (11) involved division of the pattern into a series of symmetrical curves. The results utilizing both methods are recorded in table 1.

TABLE 1  
*Areas of electrophoresis components from whey proteins*

Component	Ascending boundaries				Descending boundaries	
	A <sub>1</sub> Salt-acid		B Salt-lyophilized		Smith	Deutsch
	Tiselius & Kabat	Svedberg & Pedersen	Tiselius & Kabat	Svedberg & Pedersen	*	-
	% of total area					
1	3.0 ± 0.1**	2.7 ± 0.1	7.4 ± 0.2	7.2 ± 0.3	6	} 10.2
2	7.1 ± 0.1	6.8 ± 0.3	4.1 ± 0.1	3.4 ± 0.4	4	
4	15.8 ± 0.1	12.9 ± 0.1	17.9 ± 0.2	16.9 ± 0.4	21	18.0
4	7.4 ± 0.4	7.4 ± 0.3	8.4 ± 0.3	8.0 ± 0.4	13	20.2
5	61.8 ± 0.6	66.0 ± 0.4	56.0 ± 0.3	58.7 ± 0.7	51	48.3
6	5.0 ± 0.3	4.2 ± 0.2	6.1 ± 0.2	5.0 ± 0.3	5	3.3

\* Interpolated from values for 0.51 and 1.23% protein to 0.85% protein.

\*\* Standard error of mean, calculated from measurements on 5 projections.

Patterns obtained for the ascending boundaries were used. The electrophoresis time at which best measurements of both patterns could be made was 5,880 sec.

A micro-Kjeldahl method as described by Niederl and Niederl (8) was used to determine the nitrogen concentration of each of the samples. Protein concentrations were calculated using a conversion factor of 6.38.

## RESULTS AND DISCUSSION

The electrophoretic patterns of whey proteins prepared by either method contained six electrophoretically distinguishable peaks in relatively the same positions (fig. 2). The patterns showed no qualitative differences in the nature of

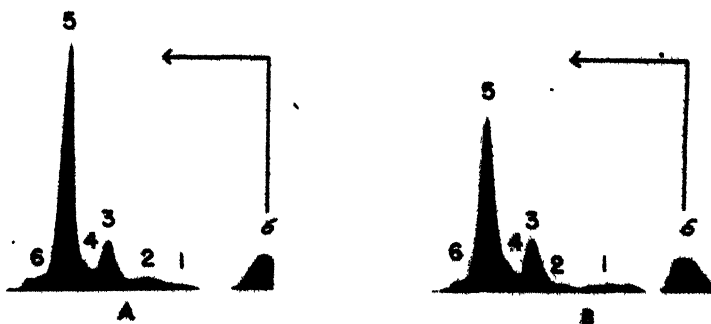


FIG. 2 Electrophoresis of whey proteins ascending boundaries. A. Salt acid 5,880 sec., 0.87% protein; B. Salt lyophilized 5,880 sec., 0.815% protein.

whey proteins prepared by either method. There were, however, quantitative differences, as shown in table 1. The concentration of component 1 by the salt-acid method was less than half the salt-lyophilized value, while the salt-acid method for component 2 gave a value nearly double that from the salt-lyophilized method. These changes were far greater than would be allowed either by the standard errors shown in table 1, or by any uncertainties of the relation between concentration and area. Smith (10) identified component 1 as euglobulin and component 2 as pseudoglobulin.

Longworth (4), working with human blood plasma, found that the two methods for measuring component areas differed by as much as 33 per cent with an average difference of 15 per cent. From values in table 1 corresponding differences in this study were 18 and 7.5 per cent, respectively.

For components 1 to 5 the data from salt-lyophilized proteins agreed with Smith's data better than the data from salt-acid proteins. This was also true of Deutsch's data except that he did not divide components 1 and 2. Only for component 6 did the data from salt-acid proteins agree somewhat better with the data of both Smith and Deutsch.

Smith used a veronal buffer of ionic strength 0.1 at pH 8.6. Deutsch used a buffer similar to the present study, except at pH 8.6 rather than 7.6. The protein concentration of his electrophoresis solution was not given. Such differences in method may account for some of the differences in peak concentrations.

According to Rowland (9) and confirmed by Harland and Ashworth, 5.64 per cent of the total nitrogen in milk is present in the form of non-protein nitrogen. In preparation of whey proteins by the salt-acid method, all of this was present in the non-protein filtrate; however, by the salt-lyophilized method, only the casein was discarded, the non-casein filtrate being lyophilized in making sam-

ple B. Thus, any non-dialyzable nitrogenous substance in the non-protein filtrate would still be present in sample B. It first was thought the increase in concentration of the euglobulin by the salt-lyophilized method could be due in part to such foreign substances. However, sample A<sub>2</sub>, as prepared for electrophoresis, produced only a minute pattern (fig. 3). This finding has been confirmed on later

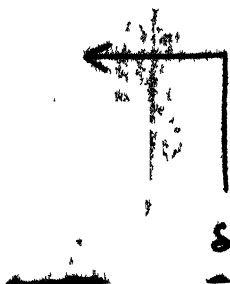


FIG. 3. Electrophoretic pattern of non protein filtrate from salt acid whey 5400 sec  $N \times 6.38/100 = 0.035$

samples concentrated to as little as 1/75th the volume of the original non protein filtrate

The original milk may be assumed to have contained 0.08 per cent nitrogen from whey proteins and 0.03 per cent non protein nitrogen. These would correspond, respectively, to 0.51 per cent protein and 0.19 per cent protein equivalent ( $N \times 6.38$ ). If none of this non protein nitrogen was dialyzable, the solution A<sub>2</sub> when concentrated to one fifteenth its original volume should have contained 0.19 per cent of 15 or 2.85 per cent protein equivalent. Analysis showed 0.035 which is only 1.2 per cent of the expected 2.85.

The electrophoretic pattern to be expected from such an assumed solution of 2.85 per cent protein equivalent should have an area nearly three and five tenths times the area of the salt lyophilized protein (B, fig. 2) containing 0.82 per cent protein. The observed pattern (fig. 3) was negligibly small.

The small observed peaks could have been due either to a small amount of non-dialyzable, non-protein, nitrogenous material or to a small amount of whey proteins that was not filtered from the non protein filtrate. It must be emphasized that these determinations were only approximate and preliminary, as no quantitative measurements of these non-protein substances have been attempted by electrophoresis. It is possible then, from this evidence, that the apparent concentration change of the 1 and 2 components could have been due to some chemical action of the HCl upon the immune lactoglobulins, resulting in a change of their electrophoretic behavior.

#### SUMMARY

1. Electrophoretic patterns were obtained for whey proteins. These proteins were prepared by two different procedures, namely, salt acid and salt-lyophilized



2. Six electrophoretically distinguishable components were split off by electrophoresis of these whey proteins.

3. No qualitative difference in whey proteins prepared by these methods was recognized.

4. The electrophoretic concentration of euglobulin by the salt-acid method was less than half the salt-lyophilized value, while for pseudoglobulin the salt-acid value was nearly double the salt-lyophilized.

5. The change of concentration could have been due to action of HCl upon the immune lactoglobulins.

6. Relative percentages of component areas from salt-lyophilized proteins agreed qualitatively with those obtained by Smith and only for one component did areas from salt-acid proteins agree better with the data of Smith and of Deutsch.

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# STALE-FLAVOR COMPONENTS IN DRIED WHOLE MILK. III. THE STEAM DISTILLATION OF STALE-FLAVOR COMPONENTS FROM STALE BUTTER OIL

R. McL. WHITNEY, KATHERINE PAULSON AND P. H. TRACY

*Department of Food Technology, University of Illinois*

In previous papers in this series (1, 2), it was demonstrated that the stale-flavor component which develops in dried whole milk upon storage could be removed from the milk powder with the butter oil. Since this component was believed to be volatile, preliminary tests were made with a conventional steam distillation apparatus which indicated that the stale-flavor component could be separated from stale butter oil by this technique. This study was undertaken to verify these observations and to develop the most satisfactory procedure.

## EXPERIMENTAL

*Preparation of the stale butter oil.* Whole milk from the University herd was condensed, dried without previous homogenization in a pilot-sized experimental spray drier and stored under varying conditions in order to have a continuous supply of stale, dried whole milk. Stale butter oil was prepared as needed from the stale milk powder by two of the extraction procedures described in a previous paper (2). In some cases, the powder was hydrated to approximately 8 per cent moisture by the dynamic method of Wilson (3) before extracting with petroleum ether, and, in the rest, the alcohol pretreatment was used. After extraction, the solvent was removed from the stale butter oil by the special technique previously reported (2). When not used immediately, the stale butter oil was stored at  $-26^{\circ}\text{C}$ .

*Steam-distillation apparatus.* While some changes were made in the apparatus throughout this study, it remained essentially a typical assembly for steam distillation under controlled pressures. The following description applies to its final form. Steam was generated in a 2-l. round-bottom flask immersed in a constant-temperature water bath. This boiler was equipped with a vacuum or pressure-release valve placed in the steam line to prevent either an explosion or the drawing back of oil from the distilling flask into the boiler. Steam from the boiler was bubbled through the melted butter oil in a 1-l. Claisen distilling flask. A thermometer, standardized against a Bureau of Standards thermometer, and a closed-end type mercury manometer were connected at the outlet of the flask to measure the temperature and pressure of the distillate at this point. The distillate then was passed through a water-cooled condenser and allowed to escape beneath the surface of the receiving liquid in a graduated cylinder maintained at as low a temperature as was practical for the particular receiving liquid. The reduced pressure in the system was obtained by a high-vacuum pump connected to the receiver through a manostat. To aid in adjusting the manostat, another closed-end type mercury manometer was inserted between it

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and the receiver. The pump was protected against moisture by means of a drying tube filled with  $\text{CaCl}_2$  with "Tell-Tale" desiccant (silica gel) as an indicator of saturation.

*Experiments.* In order that the development of the final procedure and the interpretation of the results may be followed more easily, the results of the key

TABLE 1  
*The effect of steam distillation of stale butter oil upon the distribution of stale-flavor component*

Expt. no.	Major difference from previous experiments <sup>a</sup>	Threshold value of:				
		Original stale butter oil <sup>b</sup>	Pretreated butter oil <sup>b</sup>	Residual stale butter oil <sup>b</sup>	Fresh butter oil plus distillate <sup>b</sup>	Petroleum ether soluble volatile fraction <sup>b</sup>
		(%)	(%)	(%)	(%)	(p.p.m.)
1		(6) $1.8 \pm 0.3$		(4) $2.5 \pm 0.6$	(6) $2.0 \pm 0.9$	
2	Fresh butter oil distilled	(2) $> 4.0$		(2) $> 4.0$	(2) $> 4.0$	
3	Distillation of stale butter oil under reduced pressure	(6) $2.1 \pm 0.9$		(6) $1.8 \pm 0.1$	(8) $1.8 \pm 1.4$	
4	Distilled $\text{H}_2\text{O}$ as receiving liquid	(6) $1.8 \pm 0.4$		(12) $1.8 \pm 0.4$	(2) $> 4.0$ (6) $2.0 \pm 1.3$	
5	Distillation at atmospheric pressure	(6) $1.8 \pm 0.4$		(10) $1.2 \pm 0.6$	(2) $> 4.0$ (10) $2.6 \pm 1.2$	
6	Pretreatment and distillation at reduced pressures	(6) $1.5 \pm 0.0$	(4) $1.0 \pm 0.0$	(6) $1.2 \pm 0.0$	(6) $2.4 \pm 0.5$	
7	Increased amount of distillate collected	(6) $1.7 \pm 0.1$	(8) $0.75 \pm 0.23$	(8) $1.4 \pm 1.1$ (2) $> 4.0$	(6) $1.4 \pm 0.7$	
8	No pretreatment	(6) $1.4 \pm 0.3$		(4) $1.5 \pm 0.0$	(4) $2.4 \pm 0.1$	
9	Petroleum ether used as extracting solvent	(8) $1.6 \pm 0.2$		(10) $1.8 \pm 0.3$	(2) $> 4.0$ (4) $2.6 \pm 0.4$	(2) $> 585$ (4) $380 \pm 60$
10	Anhydrous $\text{Na}_2\text{SO}_4$ used for drying ether extract	(8) $1.6 \pm 0.2$		(6) $1.3 \pm 0.3$	(6) $> 4.0$ (2) $3.0 \pm 0.0$	(6) $> 15.7$ (2) $11.8 \pm 0.0$
11	Ether-soluble volatile fraction vacuum desiccated	(6) $1.2 \pm 0.6$		(6) $2.6 \pm 1.1$	(8) $2.0 \pm 0.8$	(8) $6.2 \pm 1.9$

<sup>a</sup> Details described in full in text.

<sup>b</sup> The numbers in parentheses indicate the number of judgments. Rejected judgments are not included.

experiments are reported in table 1 and discussed individually in the following paragraphs.

In experiment no. 1, steam at atmospheric pressure (742 mm. of mercury) was passed through 60 g. of stale butter oil in the distilling flask and collected in 60 g. of butter oil prepared by melting and centrifuging freshly churned butter at 40° C. The receiver was maintained at approximately 40° C. by immersion in a constant-temperature water bath. After 200 ml. of distillate were collected in 1.75 hr., the contents of both the distilling flask and the receiver were separated and the water layers discarded. In the case of the residual butter oil in the distilling flask, it was necessary to centrifuge for 5 to 15 min. at 40° C. and 900 r.p.m. in order to break the emulsion formed during distillation. The original, residual and receiver butter oils were blended with fresh skim milk and fresh whole milk and their stale-flavor threshold values determined in the manner described previously (1). The stale flavor was detected in the receiver butter oil.

To determine whether this treatment in itself might be sufficiently vigorous to cause any flavor changes in the receiver butter oil, it was repeated in experiment no. 2 at an atmospheric pressure of 731–732 mm. of mercury with fresh butter oil replacing the stale butter oil in the distilling flask. No off-flavors were observed after distillation in either the residual or receiver butter oils when judged in the usual manner.

Since the judges comments on experiment no. 1 indicated some flavor changes in the residual butter oil which were not considered desirable, experiment no. 3 was performed under reduced pressure (68 to 74 mm. of mercury) so that the stale butter oil in the distilling flask would not be subjected to high temperatures. The temperature of the vapor before condensing did not rise above 51° C. Approximately 300 ml. of distillate were collected in 1.75 hr. Upon blending and scoring, the stale flavor again was detected in the receiver oil.

It was thought that the stale-flavor component detected in the fresh butter oil might be due to volatile catalysts from the stale butter oil which were accelerating the formation of stale-flavor components in the fresh butter oil during the distillation process. To eliminate this possibility, experiment no. 4 was performed with 60 ml. of distilled water maintained at 0° C. replacing the fresh butter oil in the receiver. After approximately 300 ml. of distillate had been collected in 2 hr. under reduced pressure (67–68 mm. of mercury) at a temperature of 34 to 38° C., fresh butter oil was added to the distillate in a separatory funnel at 40° C. and agitated for 2 min. The water layer was discarded and the butter oil blended and scored in the usual manner. The stale flavor was observed in this butter oil extract of the distillate.

Experiment no. 5 was performed in the same manner as experiment no. 4, except under atmospheric pressure (745 mm. of mercury). The stale flavor was again noted in the fresh butter oil which had been used to extract the distillate.

Since, in all previous experiments, consideration of the threshold values of the various stale butter oils indicated the possible synthesis of stale-flavor component in the residual butter oils during distillation, it was thought that this

phenomenon could be used to increase the concentration of stale-flavor component in the stale butter oil before distillation and thus improve the yield in the distillate. Preliminary tests indicated that a statistically significant decrease in the stale-flavor threshold value of the stale butter oil could be obtained by refluxing steam under reduced pressure through the butter oil for 6 hr. Therefore, in experiment no. 6, 120 g. of stale butter oil were placed in a round-bottom flask equipped with a vertical condenser and the flask was immersed in a water bath at 48 to 49° C. Then steam under reduced pressure was refluxed through this butter oil for 6 hr. The pressure was maintained at 62 to 67 mm. of mercury by a pump attached to the outlet of the reflux condenser, and the boiler temperature was held at 43 to 45° C. A 50-g. sample of the pretreated butter oil was removed, blended with fresh skim milk and fresh whole milk and scored in the usual manner. The balance of the butter oil was stored overnight at -26° C. On the following morning, it was steam distilled under reduced pressure (56-87 mm. of mercury) at 30 to 44° C. as in experiment no. 4. A statistically significant increase in the concentration of stale-flavor component was observed in the pretreated butter oil, but no improvement in the yield was obtained in the distillate.

These results seemed to indicate that the steam as it passed through the stale butter oil became saturated with the stale-flavor component under the condition of these experiments and that, in order to increase the yield, a longer distillation period would be required. Therefore, the procedure of the preceding experiment was repeated in experiment no. 7, except that the volume of distillate collected was doubled. In the pretreatment, the pressure was maintained at 66 to 70 mm. of mercury and the boiler temperature was 44.5 to 47° C. During distillation, the pressure was 66 to 72 mm. of mercury and the temperature of the vapor before condensing was 43.5 to 48° C. No statistically significant differences were observed between the threshold values of the corresponding fractions in this and the preceding experiment. A considerable variation in the threshold of the judges was noted and a number of other off-flavors were reported in these two experiments, indicating that the treatment probably was too vigorous for the purpose of this study.

In experiment no. 8, the method of experiment no. 7 was repeated without the pretreatment. The pressure was maintained at 50 to 85 mm. of mercury and the temperature before condensing was 40 to 47° C. No significant decrease in the stale-flavor threshold value for the fresh butter oil extract of the distillate could be observed when the larger volume of distillate collected was compared with the smaller volumes of previous experiments.

To determine the weight of the stale volatile fraction distilled from the stale butter oil, some modifications were necessary. In experiment no. 9, the steam distillation was performed at 54 to 62 mm. of mercury pressure with the temperature of the distillate before condensing at 40 to 42° C. The amount of distillate collected in 4.4 hr. in 60 g. of distilled water in the receiver was 287.6 g. After distillation, the contents of the condenser system were rinsed into the receiver with petroleum ether which previously had been redistilled. Then the

distillate was extracted by agitation with three successive 100-ml. portions of petroleum ether for 2 min. each. The petroleum ether extracts were combined and stored overnight at  $\sim 7^{\circ}$  C. On the following morning, the solvent was removed at room temperature by a water aspirator. The residual colorless oil, which gradually changed to a white amorphous material, weighed 878.4 mg. It then was dissolved in 50 g. of fresh butter oil at  $40^{\circ}$  C. and was blended and scored in the usual manner. The stale flavor was detected in the mixture at approximately the same threshold value as that of the fresh butter oil extracts of the distillates in the previous experiments.

The unexpectedly large weight of the petroleum ether-soluble volatile fraction obtained in the previous experiment indicated the possibility that some water had been transferred with the petroleum ether. Therefore, the procedure was modified in experiment 10 so as to dehydrate the petroleum ether extract before removing the solvent. The distillation was performed under a pressure of 52 to 62 mm. of mercury with the vapor temperature before condensing at  $39$  to  $42^{\circ}$  C. In 3 hr., 303.1 g. of distillate were collected in 60 g. of distilled water and extracted with petroleum ether as before. Then 2 g. of anhydrous  $\text{Na}_2\text{SO}_4$  were added to the extract before storing it overnight at  $\sim 7^{\circ}$  C. On the following morning, the extract was filtered to remove the  $\text{Na}_2\text{SO}_4$  before removing the solvent. The weight of the petroleum ether-soluble volatile fraction (23.6 mg.) was reduced to approximately 1/37th of that obtained in experiment no. 9, but a considerable loss of the stale-flavor component was indicated when the fraction was dissolved in fresh butter oil, blended and scored in the usual manner.

The addition of anhydrous  $\text{Na}_2\text{SO}_4$  to the petroleum ether extract therefore was omitted in experiment no. 11. The distillation was performed under a pressure of 68 to 71 mm. of mercury with the vapor temperature before condensing at  $38$  to  $48^{\circ}$  C. In 1.2 hr., 397 g. of distillate was collected in 64.3 g. of distilled water. After extraction with petroleum ether as before, the solvent was removed immediately from the extract by aspiration and the residue was stored for 60 min. in a vacuum desiccator over filter-paper strips impregnated with paraffin wax. No apparent loss of stale flavor was noted when it was dissolved in fresh butter oil, blended and scored.

#### DISCUSSION

When the results reported in table 1 are examined with respect to the volatility of the stale-flavor component with steam, the evidence is quite clear. No serious flavor changes were produced in fresh butter oil when subjected to this treatment in experiment 2. Thus the results of experiments 1 and 3 can be interpreted as indicating that either the stale-flavor component is volatile with steam at atmospheric or reduced pressures or that volatile catalysts are present. A significant increase was noted in the stale-flavor threshold value of the fresh butter oil sufficiently to produce a detectable concentration by the end of the distillation period. However, in experiments 4 and 5, when distilled water replaced the fresh butter oil in the receiver with the resulting reduction in the time of ex-

posure of the fresh butter oil to these possible volatile catalysts, no statistically significant increase was noted in the stale-flavor threshold value of the fresh butter oil containing the volatile fraction. Therefore, the stale flavor present in this fresh butter oil was not synthesized in it but rather was in the distillate from the stale butter oil in the distilling flask. Experiments 9 and 11 further this conclusion by isolating a weighable petroleum ether-soluble volatile fraction which, when dissolved in fresh butter oil and blended and scored in the usual manner, possessed a detectable stale flavor when this fraction was present in concentrations as low as 6.2 parts per million. In only one experiment (no. 10) were the majority of the judges unable to detect the stale flavor in this fraction at its highest concentration and this may possibly be attributed to the presence of the anhydrous  $\text{Na}_2\text{SO}_4$ , which may have reacted with or adsorbed the stale-flavor component. A comparison of the weights and the stale-flavor threshold values of the petroleum ether-soluble volatile fractions obtained in experiments 10 and 11, indicates that the stale-flavor component is by no means all of this fraction. The fraction secured in experiment 10, although actually the heavier, contained less stale flavor or perhaps none at all.

When these results are considered to establish the best steam distillation procedure, the evidence is not particularly clear, due to the difficulty in obtaining uniformity in the stale-flavor threshold values. However, some generally desirable conditions are indicated. In all experiments, the stale-flavor threshold values of the residual stale butter oil are lower than would be calculated from the threshold values of the original butter oils and fresh butter oils plus distillate. Also in many experiments, especially in 1 and 6, in which more vigorous treatments were used, comments made by the judges indicated the presence of other off-flavors. Therefore, distillation conditions should be as mild as possible. The time of distillation should be not more than 2 hr. The temperature of the stale butter oil should be as low as possible (40 to 50° C.). The exposure to atmospheric oxygen during distillation should be at a minimum. These conditions can be approached by performing the steam distillation in the apparatus described under approximately 70 mm. of mercury pressure with the collection of about 300 ml. of distillate. While refluxing the stale butter oil with steam under reduced pressure apparently increases the concentration of stale-flavor component in the butter oil, it is probably not desirable since no measurable increase in yield in the distillate was obtained when this was done in experiments 6 and 7 and other off-flavors may be produced by the extra treatment. Even though the stale-flavor component is not as soluble in water as in butter oil, a comparison of the stale-flavor threshold values of the fresh butter oil plus distillate fraction in the various experiments indicates that distilled water at 0° C. is as effective a receiving liquid as fresh butter oil at 40° C. The results of experiments 9 and 11 indicate that the stale-flavor component can be extracted from the distillate by petroleum ether without appreciable loss. This petroleum ether extract should constitute a very useful solution for further fractionation in the isolation of the stale-flavor component.

## SUMMARY

The possibility of separating the stale-flavor component from stale butter oil by steam distillation was investigated.

A procedure was developed by which a fraction was separated from the stale butter oil. This fraction, when dissolved in fresh butter oil and blended with fresh products to the composition of the original milk, yielded a detectable stale flavor in concentration as low as 6.2 parts per million.

This procedure involves the steam distillation of stale butter oil under a pressure of 70 mm. of mercury and at a temperature of approximately 45° C. The distillate is collected in water at 0° C. and the aqueous suspension is extracted with petroleum ether. The fraction containing the stale flavor then is obtained by evaporating the petroleum ether.

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## IMPORTANCE OF HAY QUALITY AS INDICATED BY FEEDING TRIALS WITH IDENTICAL TWIN DAIRY HEIFERS<sup>1,2</sup>

CHANDRAKANT N. KELKAR,<sup>3</sup> AND T. W. GULLICKSON

*Division of Dairy Husbandry, University of Minnesota, St. Paul*

Hay quality in relation to its feeding value for cattle has received much attention in recent years. Chemical analyses generally have shown that the higher grades of hay are lower in fiber but richer in protein, carotene and minerals than lower grades of the same kind of hay. Also, in feeding tests the higher grade hay generally has been found to be more palatable. However, only a few trials have been reported concerning the feeding value of different grades of hay for dairy heifers. Cary (2) reported that calves fed normal rations including U. S. no. 3 or poorer alfalfa hay must be given a vitamin A supplement if they are to survive. Morrison (5) states that poor quality hay supplemented with vitamin A may not be as good as similar rations containing high grade hay. Others (3, 7) have indicated that heifers cannot be raised satisfactorily on an exclusive ration of even high grade legume hay, but no harmful effects on reproduction or sexual development were reported.

### EXPERIMENTAL

The experiment was designed to compare the feeding value of U. S. no. 1 extra leafy green with U. S. no. 3 leafy brown alfalfa hay in feeding trials, utilizing two sets of identical twin heifers. In such animals the germ plasma is derived from a common zygote. Bonnier and Hanson (1) state that identical twins are at least 20 times as efficient as non-twins for use in group experiments involving growth. New Zealand workers (4) reported that one pair of identical twins is equivalent to 100 ordinary cows in studies involving milk production and 25 calves in growth studies.

Before any twins were placed on experiment the animals within each set were subjected to a rigorous comparison to establish their monozygosity. Comparisons were made for similarity in major details of color, markings, hair whorls, number of teats, shape and development of udders, nose prints, temperaments, feed intakes, weights, heights at withers, rate of gains in weight and height, blood types and hemoglobin values when fed identical rations.

The two sets of identical twin heifers selected were: T11 and T12, grade Holsteins, 287 days old, and T18 and T19, 393 days old, the offspring of a grade

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<sup>3</sup> Present address: Ramdas Peth, Nagpur, C. P., India.

Holstein dam and a purebred Red Polled sire. T11 and T12 resembled Holsteins in type and T18 and T19 closely resembled their sire in type but were black and white in color.

The experiment started on November 21, 1948, and continued for 280 days. One heifer from each pair, *i.e.*, T11 and T18, was fed U. S. no. 1 extra leafy green alfalfa hay as her sole ration and the twin sisters T12 and T19, respectively, were fed U. S. no. 3 leafy brown alfalfa hay, with T19 also being fed such amounts of a concentrate mixture as would enable her to gain at the same rate as T18. The concentrate mixture was made up of 225 lb. ground yellow corn, 300 lb. each of ground oats and ground barley, 75 lb. each of soybean oil meal and linseed meal, 15 lb. salt and 10 lb. steamed bone meal. According to the table by Morrison (5) showing the average composition and digestible nutrients in feeds, the mixture contained approximately 12.4 per cent digestible protein and 74 per cent total digestible nutrients.

The heifers when in the barn were kept in stanchions. They were fed at 7 a.m. and at 5 p.m. daily and each heifer always was fed slightly more hay than she would consume. They also had free access to clean water and a mineral mixture made up by weight of three parts steamed bone meal and one of salt. Records were kept of the amounts of feed fed to, and consumed by, each heifer daily. The heifers were turned outdoors for exercise during the day whenever weather permitted.

Composite samples were collected from both grades of alfalfa hay when fed. The results of the chemical analyses of the samples are shown in table 1. The

TABLE 1  
*Chemical analysis and digestible nutrient content of both grades of alfalfa hay fed*

Alfalfa hay	Dry matter	Crude		Ether extract	N.-free extract	Carotene	Digestible protein	Total digestible nutrients
		protein	fiber					
	(%)	(%)	(%)	(%)	(%)	(mg./100 g.)	(%)	(%)
U. S. no. 1 extra leafy green	87.2	16.1	27.0	1.8	34.5	1.9	11.9	49.7
U. S. no. 3 leafy brown	90.4	14.1	35.1	1.4	32.7	0.4	9.4	46.7

digestion coefficients reported by Morrison (5) for alfalfa hay of corresponding crude fiber contents were used in calculating the digestible protein and the total digestible nutrient contents of the two grades of hay.

Each heifer was weighed every Sunday morning before she was fed and measurements for height at withers were made every 4 wk. Records were kept of dates when each heifer showed estrum and when bred. General observations in regard to health and appearance of animals were made daily.

#### RESULTS

The weight and height at withers of each heifer at the start and at the end of the 280-day period, as well as her total gain in weight and height and average daily gain in weight, are shown in table 2. The rate of gain in weight compared

with the Ragsdale standard (6) for Holsteins also is indicated. The data show that all four heifers gained in weight at above the normal rate. Facts concerning the average weight of each heifer, average daily feed and nutrient intake, average daily nutrient requirement according to the Morrison standard (5) and

TABLE 2  
*Data concerning the weight and height at withers of each heifer*

Herd no.	Weight at		Height at withers at		Gain in weight			Total gain in height
	Start	End	Start	End	Total	Daily	Per cent of normal <sup>a</sup>	
	(lb.)	(lb.)	(cm.)	(cm.)	(lb.)	(lb.)		(cm.)
T11	517	924	113.6	130.1	407	1.45	119.4	16.5
T12	518	887	114.1	128.2	369	1.32	108.2	14.1
T18	582	951	109.7	119.2	369	1.32	112.5	9.5
T19	585	965	109.9	120.4	380	1.36	115.9	10.5

<sup>a</sup> Growth Standards for Dairy Cattle (6).

the percentage which the T.D.N. intake is of the amount required, during successive 56-day intervals and for the entire period of the experiment are presented in table 3.

The data in table 1 reveal that, pound for pound, the U. S. no. 1 extra leafy green alfalfa hay contained approximately 1.3 times as much digestible protein, 1.1 times as much T.D.N. and nearly 5 times as much carotene as the U. S. no. 3 leafy brown alfalfa hay. The U. S. no. 1 extra leafy green alfalfa hay also was considerably more palatable than the U. S. no. 3 leafy brown alfalfa hay, as indicated by the fact that the daily hay consumption by T11 was about 3.5 lb. greater than for T12.

The superiority of the no. 1 over the no. 3 grade of alfalfa also is suggested by the fact that T11 gained 38 lb. more in weight and 2.4 cm. more in height at withers than T12 during the period of the experiment. These differences, however, are small when the great difference between the nutrient intakes of the two animals is considered. T12 actually received less than 80 per cent as many pounds of T.D.N. daily (2.2 lb. less) than T11. Compared with the Morrison standard (5) T11 and T12 received 106 and 86 per cent, respectively, of their T.D.N. requirements for growth. T12 also received only about 75 per cent of the required amount of carotene. These results suggest that a more efficient utilization of nutrients received was made by T12 than by T11. Morrison (5) states, "there is even more difference in the utilization of the digested nutrients in the liberal and scanty rations than there is in the percentage digested."

Comparisons like the foregoing cannot be made between T18, fed U. S. no. 1 extra leafy green alfalfa hay, and T19, fed U. S. no. 3 leafy brown alfalfa hay, because the latter, as has been stated, was fed enough concentrates along with the hay to enable her to gain in weight at the same rate as T18. T19 consumed an average of 4 lb. concentrates daily and derived approximately 31 per cent of her T.D.N. intake from this source. The data in table 3 show that the daily

T.D.N. intake was approximately the same for both heifers and, as they made the same gains in weight, it is apparent that the nutrients supplied in both rations were equally well utilized. There was no significant difference between the two heifers in gain in height at withers.

All four heifers received an adequate supply of protein, but the rations of T12 and T19, fed the lower grade hay, were deficient in carotene; however,

TABLE 3

*Data concerning the average weight, daily feed and nutrient intakes and nutrient requirements of each heifer during successive 3-wk. periods and for the entire period of the experiment*

8-wk. period no.	Av. weight	Feed consumed		Nutrient intake			Nutrients required <sup>b</sup>			Required T. D. N. provided
		Alfalfa hay	Concen- trate	Pro- tein	T.D.N.	Caro- tene <sup>a</sup>	Pro- tein	T.D.N.	Caro- tene	
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(mg.)	(lb.)	(lb.)	(mg.)	(%)
T 11										
1	562	17.5		2.1	8.7	150	0.88	8.1	34	107.4
2	666	19.3		2.3	9.6	166	0.92	9.0	40	106.7
3	765	21.5		2.6	10.7	185	0.94	9.7	46	110.3
4	845	22.4		2.7	11.1	193	0.96	10.2	51	108.8
5	896	22.2		2.6	11.0	191	0.98	10.5	50	104.8
Av.	747	20.6		2.5	10.2	178	0.94	9.6	45	106.3
T 12										
1	552	13.0		1.2	6.1	24	0.88	8.0	33	76.3
2	634	16.7		1.6	7.8	30	0.91	8.8	38	88.6
3	722	18.2		1.7	8.5	33	0.93	9.4	43	90.4
4	798	19.6		1.8	9.2	36	0.95	9.9	48	92.9
5	855	18.7		1.8	8.7	34	0.97	10.2	48	85.3
Av.	712	17.2		1.6	8.0	31	0.93	9.3	43	86.0
T 18										
1	623	17.3		2.1	8.6	149	0.90	8.7	37	98.9
2	688	17.6		2.1	8.7	152	0.92	9.2	41	94.6
3	779	19.6		2.3	9.7	169	0.95	9.8	47	99.0
4	855	20.4		2.4	10.1	176	0.97	10.2	51	99.0
5	918	21.6		2.6	10.7	186	0.98	10.6	55	100.9
Av.	773	19.3		2.3	9.6	166	0.94	9.7	46	99.0
T 19										
1	618	14.9	1.29	1.6	7.9	27	0.90	8.6	37	91.9
2	685	15.0	3.71	1.9	9.8	27	0.92	9.1	41	107.7
3	770	15.3	4.50	2.0	10.5	28	0.94	9.7	46	108.2
4	855	13.5	5.46	1.9	10.3	25	0.97	10.2	51	101.0
5	925	10.6	5.00	1.6	8.7	19	0.98	10.6	55	82.1
Av.	771	13.9	3.99	1.8	9.4	25	0.94	9.7	46	96.9

<sup>a</sup> Carotene in concentrates not included.

<sup>b</sup> Morrison (5).

neither of these heifers exhibited symptoms of a vitamin A deficiency at any time during the period of the experiment. The physical appearance of the heifers fed no. 1 alfalfa hay was in general slightly superior throughout to that of those fed no. 3 alfalfa hay.

It is significant that there was as great a difference between the gains in weight of T11 and T18, both of which were fed no. 1 alfalfa hay, as between T11

and her twin sister T12, fed no. 3 alfalfa hay. These results illustrate how the individuality of animals selected may affect experimental results. By using identical twins this upsetting factor largely is eliminated.

The evidence is incomplete in regard to the possible effect of quality of hay fed on sexual development of heifers. The records show that T18 and T19 were sexually mature when started on experiment. T18 came in heat and was bred on December 1, 1948, and T19 was bred on December 20. Both heifers were bred to the same sire and each conceived from a single service. T11 first came in heat December 29, 1948, but T12 did not show estrum until 96 days later. After the first heat period, however, each heifer showed estrum regularly. T12 was first bred on May 17, 1949, and T11, 11 days later. Since that time both heifers were bred regularly at each heat period but neither of them conceived. No explanation other than difference in quality of hay fed is available to account for the delay in appearance of estrum in T12.

#### CONCLUSIONS

The U. S. no. 1 extra leafy green alfalfa hay was more palatable, richer in digestible nutrients and carotene and, when fed to heifers, produced slightly more rapid gains in weight and in height at withers than U. S. no. 3 leafy brown alfalfa hay, but at a higher cost in nutrients per unit of gain.

The value of using identical twins in experiments comparing the nutritive value of feeds is indicated.

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# THE NUMBER OF PROVED SONS NECESSARY TO EVALUATE THE TRANSMITTING ABILITY OF A DAIRY SIRE <sup>1, 2</sup>

W. E. WASHBON<sup>3</sup> AND W. J. TYLER  
*West Virginia University, Morgantown*

Breeders of dairy cattle constantly are looking for more aids to use in selecting future herd sires. Washbon (2, 3) has advocated the use of the performance of a sire's proved sons (based on Dairy Herd Improvement Association records) as a basis for evaluating the transmitting ability of the sire and has suggested selection of bulls whose paternal brothers have sired daughters that produce more butterfat than their dams at a relatively high level of production.

The reliability of selecting bulls by this method depends upon how well the later proved sons of a sire repeat the performance of their earlier proved paternal brothers. This paper reports the results of a study of the correlation between the daughter performance of varying numbers of first proved and later proved paternal brothers.

## EXPERIMENTAL PROCEDURE

One hundred seventy-four Holstein sires with eight or more DHIA proved sons were studied to determine the least number of proved sons necessary to estimate most accurately the performance of those to be proved later. The data included: (a) average butterfat production of the proved sons' daughters, (b) average differences of daughters' production as compared to their dams and (c) the per cent of proved sons that maintained or increased butterfat production of the herds in which they were used. The average performance of the first three to ten proved sons, respectively, was compared with the performance of the following five and ten sons.

The essential information about each proved son of the 174 Holstein sires studied was punched on International Business Machine cards. These cards, approximately 3,000 in number, then were sorted by sires either in actual sequence of proof, if known, or in sequence of registration. The cards were arranged so as to obtain the listing and totals of the first three sons, the next five sons and the next ten sons for each of the 272 sires. The cards then were rearranged by placing the first four sons in the first group, to be compared with the following five and ten sons. This was repeated for the first five, six, seven, eight, nine and ten sons where sufficient numbers made comparisons possible.

The essential data and the averages of each group and of the groups with

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<sup>3</sup> Now Cattaraugus County Agricultural Agent, Salamanca, New York.

which each was to be compared were computed and punched upon summary cards. The summary cards were used to obtain listings of comparative performance of associated groups of sons. The summary cards again were used to obtain sums of squares and cross products for statistical analysis of the data.

A difference in the average number of dam-daughter comparisons between the first proved sons and those proved later might affect the conclusions drawn from a comparison of two groups of paternal brothers. A study involving 118 Holstein sires with 1,782 proved sons indicated that there was no appreciable difference in the average number of dam-daughter comparisons between groups of the data to be studied.

A consistent but slight tendency for the better sons of a sire to be proved more promptly than the poorer ones was indicated by a consistently higher fat level and daughter fat difference of first proved sons arranged by actual order of proof as compared to the same sons arranged in sequence of registration. The extent of this influence was not considered sufficient to affect materially the investigation.

#### RESULTS

The average butterfat produced by the daughters of the first three to ten proved sons was 386 lb., which was 10 lb. less than the average production of the daughters of the next five or ten proved sons (Table 1). This greater produc-

TABLE 1

*Means and regression coefficients (b) of mean daughter fat of later proved sons upon the means of three, five and ten sons proved earlier*

First proved sons		Next 5 sons		Next 10 sons	
Number	Mean	Mean	b	Mean	b
3	385	391	0.66	395	0.55
5	387	394	0.62	393	0.59
10	384	400	0.60	395	0.37

tion of the later proved sons' daughters is ascribed largely to the gradual improvement of economic conditions from 1930 to 1948 which favored daughters of the later proved sons. The standard deviation of the average daughter fat of the groups of proved sons ranged from 40 lb. of fat with three sons in the group to 25 lb. when the group contained from six to ten sons.

The correlation coefficients between average daughter butterfat of the first proved Holstein sons and those proved later were statistically significant and ranged from 0.40 to 0.65, as shown in table 2. Correlations decreased as the average daughter butterfat of the first eight, nine and ten proved sons was compared to the performance of the daughters of the next ten. This was the opposite of normal expectation, since a comparison involving larger numbers would be expected to show higher correlations than smaller numbers. This trend did not exist when comparing the first three to ten proved sons, respectively, with the next five sons. Weighted average correlations (1) of sons' daughters' average butterfat between the first three to ten proved sons, inclusive, and the next

TABLE 2  
*Correlation coefficients between average performance of first proved sons and those proved later of Holstein sires*

First sons proved	Correlation coefficients between butterfat level of the daughters of first and later proved sons				Correlation coefficients between fat difference of the daughters of first and later proved sons				Correlation coefficients between per cent of sons plus of first and later proved sons			
	No. of com- parisons	Next 5 sons	No. of com- parisons	Next 10 sons	No. of com- parisons	Next 5 sons	No. of com- parisons	Next 10 sons	No. of com- parisons	Next 5 sons	No. of com- parisons	Next 10 sons
3	174	0.50**	72	0.62**	174	0.30**	72	0.31**	174	0.15*	72	0.12
4	148	0.46**	59	0.51**	148	0.19*	59	0.28*	148	0.08	59	0.31*
5	129	0.45**	52	0.56**	129	0.10	52	0.35**	129	0.00	52	0.32*
6	103	0.53**	46	0.65**	103	0.35**	46	0.40**	103	0.17	46	0.31*
7	87	0.50**	41	0.54**	87	0.29**	41	0.32*	87	0.04	41	0.25
8	72	0.48**	34	0.49**	72	0.36**	34	0.24	72	0.12	34	0.13
9	59	0.48**	29	0.45*	59	0.30*	29	0.04	59	0.25	29	0.01
10	52	0.64**	27	0.40*	52	0.36	27	0.04	52	0.24	27	0.04
Weighted av. $\bar{r}$		0.50**		0.55**		0.26**		0.28**		0.11		0.21

\*  $0.01 < P < 0.05$  = Significant.

\*\*  $P < 0.01$  = Highly significant.



five and next ten sons were computed and are given in the bottom line of table 1.

The regression coefficients of mean daughter fat of later proved sons upon those proved first ranged from 0.32 to 0.85, most of them being in the range 0.45 to 0.60.

The correlation coefficients between the mean daughter fat difference of the first proved sons and those proved later ranged from 0.04 to 0.40 and are shown in table 1. The weighted average correlation between the first three to ten proved sons, inclusive, and the next five sons was 0.26, while a weighted correlation of 0.28 was obtained in the comparison between the first three to ten proved sons and the performance of the next ten sons.

The average per cent of sons maintaining or increasing production does not change materially as more sons are proved. There was some indication of a trend toward a lower percentage as more than 15 sons were proved.

The correlation coefficients between the average per cent sons plus of the first proved sons and those proved later are given in table 1. The average weighted correlation between the first three to ten sons, inclusive, and the next five sons was 0.11, while a correlation of 0.21 was obtained between the first group and the next ten sons.

#### DISCUSSION

The variations between groups of sons of the same sire are a result of sampling of the sire's inheritance, differences in transmitting abilities of the dams of the sons, differences in the average inheritance of the cows to which the sons are mated, and differences in temporary or permanent environments affecting the herds in which the sons were used. A group of ten sons of a sire might be mated to dams that averaged as much as 75 lb. of fat above or below the dams to which another ten sons were mated. One group of sons could, by chance, be mated to dams of better inheritance than another group. These extreme conditions were rather uncommon within the data studied but did exist in a few instances.

There was some indication that the differences in the inheritance of the dams of the sons contributed to this variation. Among the 154 sires with sons arranged by order of registration number, there were several instances in which a higher proportion of first born sons increased or decreased production. This was followed by the opposite performance in later proved sons. It might happen that this relationship would occur purely by chance, although this is not to be expected. Partial explanation might lie in the often-followed policy of mating a young bull only to the daughters of an older sire at the farm. If the older sire possessed outstanding inheritance, the sons from such matings might be superior. As the young bull grew older, matings with the older females in the herd might give his later sons poorer inheritance than his first born sons because these new mates could be cows more representative of the herd average. The exact opposite of the situation is as likely in which the older sire was an outstandingly poor one.

The correlation coefficients indicated that the progeny performance of four or five proved sons was fairly reliable as a measure of a sire's transmission to future sons. When the first eight, nine and ten sons were compared with the next ten sons, the correlations were smaller than the correlations between the first three to seven sons and the next ten sons. However, the sampling error is large because of the small number of sires that had 18 or more sons, and may explain the differences observed here.

The method of averaging correlation coefficients was adopted because of the consistent nature of relationships from group to group. The weighted average correlation should give a slightly more reliable statistic than any individual correlation. The degrees of freedom for the weighted statistic will not be much larger than the largest number of degrees of freedom for any of the correlations that were averaged.

The correlation between one group of sons and the next group of sons by the same sire is a measure of the genetic similarities between the two groups plus the fact that the bulls in the two groups may have been used in herds with similar environment. Sires with proved sons performing at relatively high levels of production had groups of sons that fluctuated from that high average as much as groups of sons from mediocre sires varied from a mediocre average. When these son-group fluctuations were charted it was clear that the groups of sons from the superior sires fluctuated at a high level, the groups of sons from the mediocre sires varied around the breed average and the groups of sons from the poor sires fluctuated at levels below breed average. The pronounced tendency for groups of sons from superior sires to vary at consistently higher-than-average levels might allow more certain identification of desired inheritance provided the effects of similarities in herd environments for the sons of such sires can be evaluated. If stratification of herd environments is inherent in these data, then consideration of this factor should be taken into consideration if breeders are to select bulls on the basis of the performance of the bull's paternal brothers. This problem of evaluating the relative importance of herd environment and heredity needs further study.

#### SUMMARY

A total of 174 Holstein-Friesian sires with eight or more D.H.I.A.-proved sons was studied to determine the least number of proved sons necessary to estimate accurately the performance of those to be proved later. The results showed that the average butterfat production of the sons' daughters increased as more sons were proven, a tendency that also was reflected in greater daughters' fat difference as compared to their dams. This increase probably was due more to environmental than genetic causes.

Highly significant correlations ( $r = 0.46$  to  $0.62$ ) indicated that the average butterfat production of the daughters of the first three or four sons was nearly as accurate as data on more sons in estimating the average butterfat production of the daughters of the next five or ten proved sons.

Similarly, the significant correlations ( $r = 0.19$  to  $0.31$ ) for the sons' daughters' average increase or decrease in butterfat production from their dams

indicated that data on the first three or four proved sons were nearly as accurate as data on a larger number in predicting what might be expected from the next five or ten proved sons in this respect.

The correlations for per cent sons improving production were lower than those for fat level or fat difference. Figures on the first three or four sons were as accurate as data on a larger number of sons in predicting percentage of improvers in the next five or ten proved sons.

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## FLAVOR DETERIORATION ASSOCIATED WITH THE LIPID PHASE OF WHOLE MILK POWDER<sup>1</sup>

ARTHUR T. MUSSET,<sup>2</sup> STUART PATTON AND CHESTER D. DAHLE  
*Department of Dairy Husbandry, Pennsylvania Agricultural Experiment Station,  
State College*

Whole milk powder readily undergoes flavor deterioration, either during manufacture or shortly thereafter. This flavor defect is intensified with increased length of storage or elevated storage temperature. Definition of the defect includes such terms as "typical of whole milk powder," "heated," "like coconut," "stale" and various others. This defect should not be confused with oxidized flavor of dairy products, since it is detectable in whole milk powders of best quality long before true oxidized flavor normally is evident. Milk powder in which the milk fat has been replaced with a suitable vegetable fat appears to be free of this flavor (8).

Bailey (3) states, "Flavor reversion in fats is probably defined most satisfactorily as the appearance of objectionable flavor from less oxidation than is necessary to produce true oxidative rancidity." He points out that the amount of oxygen necessary to produce flavor deterioration in partially hydrogenated lard was only one-fiftieth of that necessary to produce (oxidative) rancidity. Bailey further believes "the respective phenomena of flavor reversion and rancidification are associated with amounts of oxidation so different in degree that it appears hardly proper to consider them more than casually related."

Some preliminary investigations (11) of flavor change in dry butterfat during storage indicated great similarity between the flavor developing in this medium and that produced in dry whole milk during its manufacture or shortly thereafter. West (15) observed this phenomenon when aged dry butterfat was re-emulsified in fresh, pasteurized skim milk. In addition, he found that the use of antioxidants and nitrogen packing, either together or separately, only delayed rather than prevented the onset of the "typical whole milk powder" flavor defect in dry butterfat stored as such. The development of this flavor in stored dry butterfat is catalyzed by heat and appears to resemble the mechanism of flavor deterioration in many other fats and oils.

Ashworth (1) reported that heating of the skim milk and cream separately prior to drying showed some promise of improving the shelf life of the resulting powder. Less flavor change was noted in powder made from concentrated skim milk receiving high heat treatment and cream receiving low heat treatment. A patent issued earlier to North and Alton (12) claims that the keeping quality of whole milk powders are enhanced if the cream is not subjected to condensing.

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<sup>2</sup> Present address: Beatrice Foods Co., Chicago, Ill.

Whitney and Tracy (16) recently have isolated a stale flavor component from dry whole milk which appears to be concentrated in the milk fat phase. These authors indicate that stale flavor develops only after some period of storage, whereas the flavor of interest in this report is that exhibited immediately after manufacture or a very short storage period.

This investigation was conducted to determine the effect of different fats on the initial and storage flavor qualities of dry whole milk. Secondly, a study of the storage characteristics of certain fractions of butter fat was made to determine which fractions are most concerned in the flavor deterioration of whole milk powders.

#### EXPERIMENTAL

*Dry whole milk.* In order to study the contribution of the lipid phase of dry whole milk to flavor deterioration occurring at the time of manufacture or shortly thereafter, samples of dry whole milk were prepared utilizing the following fats: (a) Control (whole milk with fat untreated); (b) partially hydrogenated coconut fat (melting point 76° F.); (c) dry butterfat; and (d) a combination of 75 per cent partially hydrogenated cocoanut fat and 25 per cent dry butterfat. A quantity of high quality mixed herd milk was obtained from the college creamery and treated as follows: A portion of the milk was removed, standardized to 3.4 per cent fat, forewarmed to 170° F. for 30 min. and concentrated to approximately 36 per cent total solids. Upon completion of evaporation, the condensed milk was homogenized at 2500 p.s.i. through a single stage homogenizer, cooled to approximately 40° F. and held for drying the following day.

The remainder of the milk was centrifugally separated. The skim milk portion was forewarmed to 170° F. for 30 min. and condensed to approximately 29 per cent total solids, cooled and divided into three equal lots for addition and emulsification of fats. To one lot of the condensed skim enough partially hydrogenated coconut fat was added to give a fat/snf ratio equal to that of the control whole milk. Dry butterfat for addition to the second lot of condensed skim milk was prepared from the cream by a conventional method. Briefly, this consisted of pasteurizing (155° F. for 30 min.), cooling and churning the cream. The resulting butter was thoroughly washed, melted and rewashed several times with warm water. After the final washing, the wash water was removed by passing the oil-water mixture through an International Harvester separator. A mixture of 75 per cent partially hydrogenated coconut fat and 25 per cent butterfat was added to the third lot of condensed skim milk. The various fats were emulsified in the condensed skim milk by homogenization through a single stage at 2500 p.s.i.

The experimental batches of milk were dried in a Rogers chest-type spray drier which had four no. 72 atomizing nozzles and a capacity of 140 lb. of powder per hour. The milk was atomized at a pressure of 2500 p.s.i. and the outlet temperature of the drying chamber was approximately 186° F.

The powders were sifted and weighed into no. 1 tin containers. The tins

then were placed in a large container and subjected to a vacuum of 28 in. for 18 hr. At the end of this period, the cans were removed and sealed individually by means of an American Can Co. machine designed for automatic sealing under vacuum. The samples of powder were stored at room temperature. The fat ( $27.85 \pm 0.40$  per cent) and moisture ( $2.33 \pm 0.25$  per cent) content of all samples were relatively uniform.

Since there appeared to be no suitable chemical tests for measuring the flavor changes under consideration, and in view of the fact that several groups of investigators (4, 5, 6, 7, 9 and 10) have found organoleptic procedures the most useful for detecting flavor deterioration, such procedures were employed for evaluating flavor in these experiments. Five experienced judges were used at each taste session. Only one judge at a time was permitted to examine the samples. The identities of the samples were unknown to the judges. Criticisms of the samples were classified as slight, definite, pronounced and very pronounced. Flavor evaluations of the samples were made following manufacture and at monthly intervals thereafter.

*Dry butterfat fractions.* In an attempt to clarify the nature of flavor deterioration in dry butterfat, storage studies were conducted on various high and low melting butterfat fractions. The Winterization method of obtaining the desired fractions was selected over solvent crystallization as being least rigorous in treatment of the fat from a flavor viewpoint. This method has had commercial application in the vegetable oil and beef tallow industries for many years as a means of fractionating fats.

Dry butterfat was prepared from fresh sweet cream obtained from high quality milk of mixed herds on winter rations. The method of making the dry butterfat previously is described in this report. Prior to fractionation the butterfat was dried under vacuum to a moisture content of less than 0.1 per cent. After drying, the butterfat was divided into four equal lots which were treated as follows: Lot one was used as the control dry butterfat. The remaining three lots were adjusted to 32, 50 and 70° F., respectively, and allowed to crystallize for 24 hr. Upon completion of the crystallization period, each lot of liquid-solid mass was placed in a Carver hydraulic laboratory press for separation. The pressure on the mass was increased gradually by means of an hydraulic ram to 8000 p.s.i. and held at that pressure for several minutes. The liquid was collected and solids removed from the press for weighing and calculation of the per cent yield. Samples of the control butterfat along with each of the various liquid and solid fractions were air-packed in brown glass bottles for storage at room temperature. The melting points were determined on the fresh samples by the capillary tube method (2). Iodine numbers were ascertained by the Rosenmund-Kuhnhehn method (14).

By use of a hand homogenizer and fresh pasteurized skim milk, the samples of fat were re-emulsified on the basis of 4 per cent butterfat. Procedures for organoleptic evaluation of the re-emulsified samples were the same as those previously given for dry whole milk.

## DISCUSSION AND RESULTS

From a beverage standpoint, dry whole milk has certain inherent flavor defects. Although such defects may vary with manufacturing and storage conditions of the powder, they are evident in freshly-made dry milk of highest quality. The results of this study indicate that these initial flavor defects of dry whole milk have their origin, in part at least, in the lipid phase of the powder. The data in table 1 show clearly that a portion of the inherent flavor defects of whole

TABLE 1

*The effect of storage on the flavor of dry whole milks<sup>a</sup> made with various fats*

Type of fat	Flavor after storage in months						
	0	1	2	3	4	5	6
Control	+ milk powder	++ milk powder	++ milk powder	++ milk powder	+++ milk powder	+++ milk powder	+++ milk powder
Coconut fat	bland	bland	no criticism	no criticism	flat	flat <sup>b</sup>	flat
Dry butterfat	++ milk powder	++ milk powder	+++ milk powder	++++ milk powder	++ oxidized	++ oxidized	+++ oxidized
75% coconut fat 25% dry butterfat	bland	no criticism	no criticism	no criticism	flat	flat	stale

<sup>a</sup> Double vacuum packed in no. 1 tins and stored at room temperature.

<sup>b</sup> Resembling raw potato flavor.

Code: + slight; ++definite; +++pronounced; ++++ very pronounced.

milk powder do not develop when part or all of the butterfat is replaced with hydrogenated coconut fat. These findings were confirmed by flavor data obtained independently at the Quartermaster Food Acceptance Laboratories on the same samples (13). It was observed further that the odor in the drying chamber following the processing of samples containing pure butterfat was typical of that associated with whole milk powder, whereas the milk containing hydrogenated coconut fat imparted little or no odor to the chamber.

The experiments dealing with flavor deterioration in dry butterfat, stored as such, also tend to support the observation that the lipid phase of dry whole milk is responsible for a portion of the characteristic flavor defect in the powder. The aged butterfat, when reconstituted in fresh skim milk, imparted a flavor very similar to that of dry whole milk (table 2). Certain of the taste panel members had difficulty in distinguishing between aged dry butterfat and dry whole milk when these products were reconstituted as beverage milk. The dry butterfat experiments also show that fat fractions obtained by the hydraulic expression of crystallized butterfat vary in their stability to storage deterioration. The solid fractions appear to be relatively stable in this respect, whereas the liquid fractions deteriorate rapidly. That the liquid fractions undergo considerable change during storage is borne out by significant variations in their iodine numbers (table 3), as well as by their flavor on reconstitution in fresh skim milk. The solid fat fractions seem to afford some protection of an antioxidant type to

TABLE 2

*The effect of storage<sup>a</sup> on the flavor of fat fractions obtained by the hydraulic expression of dry butterfat at various temperatures*

Fractions	Age of samples					
	Fresh	2 wk.	1 mo.	2 mo.	3 mo.	4 mo.
Control	no criticism	no criticism	+ milk powder	++ milk powder	+++ milk powder	+++ milk powder
32° F. Liquid	++ oxidized	+++ oxidized	+++ oxidized			
32° F. Solid	no criticism	no criticism	no criticism	+ milk powder	++ milk powder	++ milk powder
50° F. Liquid	+ milk powder	++ oxidized	+++ oxidized			
50° F. Solid	no criticism	no criticism	no criticism	no criticism	+ milk powder	+ milk powder
70° F. Liquid	+ milk powder	++ oxidized	+++ oxidized	+++ oxidized	+++ oxidized	
70° F. Solid	no criticism	no criticism	no criticism	+ butter-scotch	+ butter-scotch	+ milk powder

<sup>a</sup> Air packed in brown glass bottles at room temperature.

Code: + slight; ++ definite; +++ pronounced; ++++ very pronounced.

the liquid fractions, as evidenced by the relative stability of the control dry butterfat and a secondary control made by combining liquid and solid fat fractions immediately following expression.

TABLE 3

*Some properties of fat fractions obtained by the hydraulic expression<sup>a</sup> of crystallized dry butterfat at various temperatures*

	Control	32° F.		50° F.		70° F.	
		Liquid	Solid	Liquid	Solid	Liquid	Solid
Melting Point (° F.)	86-89	46-53	91-95	50-53	98	53-56	109
Yield (%)		7	93	14	86	63	37
Iodine Numbers							
Fresh	33.0	36.2	31.9	39.9	29.9	36.3	25.2
4 mo.	31.5	34.5	30.9	37.7	29.3	34.6	24.7
Net change	-1.5	-1.7	-0.8	-2.2	-0.6	-1.7	-0.5

<sup>a</sup> 8000 p.s.i. pressure used.

#### SUMMARY AND CONCLUSIONS

Studies of inherent and initial flavor changes produced during manufacture and early storage of dry whole milk indicate that deterioration of the lipid phase accounts, in part at least, for such changes. Milk powder prepared with hydrogenated vegetable fat was lacking in certain of the flavors normally present in dry milks containing butterfat. Further, aged dry butterfat upon reemulsification in fresh skim milk was observed to impart a flavor very similar to that typical of fresh dry whole milk of good quality. These findings were considered good evidence that dry butterfat may undergo certain initial changes in flavor prior to the onset of oxidative rancidity. Such changes in dry butterfat appear



to be catalyzed by heat and the presence of oxygen; however, they may take place at relatively low temperatures and oxygen levels during longer periods of time.

Fat fractions, obtained by hydraulic expression of butterfat crystallized at various temperatures, showed consistent variations in keeping quality. The solid fat fractions were all relatively stable with respect to flavor deterioration during storage, whereas all the liquid fractions deteriorated rapidly. It is suggested that preliminary deterioration of the low melting portion of butterfat may be responsible for the flavor changes observed in connection with dry whole milk and stored dry butterfat in this investigation.

The viewpoints and conclusions of this paper are not to be construed as approval of the authors for the use of substitute fats in dairy products. Substitute fats were used in these experiments as an academic approach to the question of flavor deterioration of a lipid nature in dry whole milk.

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# THE USE OF DEHYDRATED FORAGES IN DAIRY CATTLE RATIONS.

## I. GRAIN SUBSTITUTION WITH FINELY GROUND MATERIAL<sup>1</sup>

E. B. HOPE, R. E. ERB, T. H. BLOSSER, U. S. ASHWORTH AND A. O. SHAW<sup>2</sup>  
*Division of Dairy Husbandry, Washington Agricultural Experiment Stations,  
State College of Washington, Pullman*

Numerous feeding trials have been conducted with milking cows and growing heifers using many different kinds of dehydrated forages. In these trials dehydrated forages clearly have shown a superiority for both growth and milk production over sun-cured forages harvested from the same field. Most of the earlier work on the nutritive value of dehydrated forages has been very well reviewed by Huffman (4) in 1939 and by Watson (13) in 1948.

Several workers have compared the value of dehydrated forages as a partial-to-complete replacement of concentrate mixtures for milk production. Woodman *et al.* (15), using dried grass protein cake, replaced all of the concentrate mixture and observed satisfactory milk and fat production. Watson (12) replaced the concentrate ration of five cows for 14 days with dehydrated grass without too much decline in production. Newlander (9) and Knott and Hodgson (5) replaced a portion of the concentrate mixture with dehydrated grass in change-over type experiments with short feeding periods without observing appreciable declines in milk production. Watson and Ferguson (14) fed an average of 8 lb. of dehydrated grass in meal form as a partial replacement for concentrates, using 5-wk. experimental periods. There was no statistical difference between milk, fat and solids-not-fat production when compared with controls. These workers further observed that the cows would eat no more than 8 to 10 lb. daily of this powdered material. In this connection, Cole and Mead (3), feeding finely ground alfalfa as the sole roughage, observed diminished rumination, depraved appetite and recurrent bloat. Camburn (2) replaced a part to all of a 20 per cent protein grain mixture with dried young grass, in a reversal type experiment with 3-wk. experimental periods, without observing any decline in milk production.

The purpose of the experiment herein reported was to test the grain replacement value of a commercially prepared lot of dehydrated grass with high-producing dairy cows at 15, 30 and 45 per cent levels of grain replacement in a change-over type of experiment.

### EXPERIMENTAL

**Animals.** Twenty high-producing cows were divided into four groups consisting of three Holstein, one Jersey and one Guernsey per group. The age,

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stage of lactation, breeding date and production of each cow is shown in table 1. Cow 269 from group 2 and cow 300 from group 4 were removed from the experiment at the end of the first period and were replaced by cow 232 and cow 212, respectively. Cow 269 was suffering from rumen atony and possibly other undetermined ailments, and cow 300 died from a foreign body of long standing. Neither incident can be attributed to this experiment.

TABLE 1

*Age, date of freshening, productive level, date of breeding and group assignment of each cow used*

Group	Herd no.	Breed	Age	Freshening date	4% F.C.M. for first transition week	Date bred
			(yr.)		(lb.)	
1	2124	J	2.5	3-26-48	223.0	11- 6-48
	3058	G	3	4-13-48	261.6	11- 8-48
	237	H	7	4-29-48	449.7	
	256	H	5.5	5-25-48	453.2	8-19-48
	274	H	4	10- 3-47	306.1	
Av.					338.7	
2	2125	J	2.5	3-27-48	188.7	12-24-48
	3900	G	4	6- 6-48	356.1	11-24-48
	207	H	9	10- 1-47	315.3	
	296	H	2.5	4-16-48	295.7	11- 7-48
	269 <sup>a</sup>	H	4.5	3-28-48	336.5	
	232 <sup>b</sup>	H	7.5	7- 1-48	371.9	
Av. <sup>c</sup>					305.5	
3	2122	J	3	3- 4-48	201.4	11- 2-48
	3903	G	4	4-12-48	269.2	
	297	H	2.5	4-24-48	296.0	7-13-48
	249	H	6.5	4-23-48	440.2	
	253	H	6	5-20-48	377.2	11- 4-48
Av.					317.8	
4	2123	J	2.5	3-31-48	202.4	12- 6-48
	3902	G	4	6-26-48	343.1	11-26-48
	264	H	5	5-23-48	443.8	8- 7-48
	300 <sup>a</sup>	H	2.5	4- 1-48	276.0	
	212 <sup>b</sup>	H	8.5	6- 4-48	293.3	11-15-48
	231	H	7.5	5-21-48	398.6	8-12-48
Av. <sup>c</sup>					336.2	

<sup>a</sup> Removed from experiment at end of first period.

<sup>b</sup> Placed on experiment beginning of second period.

<sup>c</sup> Cows under footnote (a) not included in the group average.

**Rations.** The roughages used consisted of good quality, chopped alfalfa hay and a grass-legume silage. The dehydrated grass was a mixture of orchard grass, ladino clover, alsike clover and timothy. This mixture was seeded 2 yr. previous to dehydration on land which had been treated with 2 tons of limestone and 100 lb. of treble superphosphate per acre. The grasses were harvested at an average height of 10 in. and dehydrated in an Arnold Dehydrator at approximately 1,700° F. This material was fed in finely ground form.

The grain mixture used consisted of 200 lb. millrun, 400 lb. ground cull peas, 600 lb. ground oats, 300 lb. ground barley, 200 lb. ground whole corn, 300 lb.

linseed oil meal, 20 lb. iodized salt, 20 lb. steam bone meal, 4 oz. type 9-F irradiated yeast and 2 oz. of manganese sulfate, feeding grade. The average analyses of feeds as fed are shown in table 2.

*Procedure.* Silage was fed at the rate of 3 lb. for each 100 lb. of body weight. Grain was fed at the rate of 1 lb. for each 3 lb. of 4 per cent fat-corrected milk produced daily. The amount of silage and grain fed was adjusted weekly ac-

TABLE 2  
*Analyses of feeds on a moisture basis as fed*

	Alfalfa haya	Silage <sup>b</sup>	Grain <sup>c</sup>	Dehydrated grass <sup>d</sup>
	(%)	(%)	(%)	(%)
Crude protein	14.0	3.8	16.8	14.7
Ether extract	1.7	1.1	3.6	3.9
Ash	8.3	3.4	5.5	6.5
Crude fiber	34.7	13.1	9.3	21.4
Nitrogen free extract	32.9	13.1	55.6	44.4
Dry matter	91.5	34.5	90.8	90.8
Total digestible nutrients	49.1 <sup>e</sup>	20.3 <sup>f</sup>	71.3 <sup>f</sup>	66.8 <sup>e</sup>

<sup>a</sup> Av. of 8 samples

<sup>b</sup> Av. of 6 samples.

<sup>c</sup> Av. of 2 samples.

<sup>d</sup> Av. of 4 samples.

<sup>e</sup> Determined by digestion trial using 3 sheep.

<sup>f</sup> Calculated using Morrison's tables. (8)

cording to body weight changes and milk production. Hay was fed *ad libitum*. Dehydrated grass legume (hereafter referred to as dehydrated grass) was used to replace grain at three different levels. The four rations fed were as follows: (a) control—no grain replacement; (b) 15 per cent of grain replaced with dehydrated grass; (c) 30 per cent of grain replaced with dehydrated grass; (d) 45 per cent of grain replaced with dehydrated grass. These were straight

TABLE 3  
*Design of the experiment*

Ration	July 10– Aug. 21	Aug. 28– Oct. 9	Oct. 16– Nov. 27	Dec. 4– Jan. 15
	Period 1	Period 2	Period 3	Period 4
Control	Group 1	Group 4	Group 3	Group 2
15% of grain replaced	Group 2	Group 1	Group 4	Group 3
30% of grain replaced	Group 3	Group 2	Group 1	Group 4
45% of grain replaced	Group 4	Group 3	Group 2	Group 1

weight replacements. The cows were housed in a conventional tie-stall barn and were allowed approximately 2.5 hr. exercise per day in a paved yard. The mangers were equipped with metal partitions so that the feed intake of each cow was recorded accurately. All refused feed was collected and weighed. Composite samples of weighbacks for each cow were accumulated over each experimental period and analyzed.

As shown in table 3, the feeding trial was divided into four 6-wk. periods with a 1-wk. transition period between each change-over. The experiment ran a total of 28 wk. from July 3, 1948, to January 15, 1949. All cows were milked three times daily at regular 8-hr. intervals. Grain, dehydrated grass and hay were fed three times daily and silage twice daily. Feed analyses were made according to procedures outlined by the Association of Official Agricultural Chemists, sixth edition (1).

Feed consumption in terms of total digestible nutrients was calculated for each cow by weeks, using the results of digestion trials for the alfalfa hay and dehydrated grass and using digestion coefficients listed in Morrison's tables (8) for the grain and silage. Since moisture in weighbacks varied from cow to cow, it was necessary to take one sample per cow per period and determine the difference in weight of the sample at collection time as compared with the weight when the sample was allowed to air dry. The weighbacks for each cow were corrected accordingly. The total digestible nutrients then were calculated from an assumed ration of two-thirds hay and one-third silage, using Morrison's (8) digestion coefficients for hay and silage as applied to the chemical analyses as determined. Statistical analyses were made essentially as outlined in Snedecor (10).

The average chemical analyses and standard errors of weighbacks by rations are shown in table 4. There were no appreciable differences in average analyses

TABLE 4  
*Average chemical analyses and standard error of weighbacks, by rations on a moisture per sample basis*

	Rations			
	Amount of grain replaced with dehydrated grass			
	0% <sup>a</sup>	15% <sup>b</sup>	30% <sup>a</sup>	45% <sup>b</sup>
	(%)	(%)	(%)	(%)
Dry matter	92.7 ± 0.48	93.2 ± 0.36	93.3 ± 0.32	91.4 ± 0.30
Crude protein	10.5 ± 0.31	10.5 ± 0.34	10.7 ± 0.88	12.0 ± 0.37
Ether extract	1.7 ± 0.10	1.6 ± 0.06	1.6 ± 0.07	1.7 ± 0.09
Ash	8.2 ± 0.19	8.0 ± 0.19	8.0 ± 0.20	8.0 ± 0.70
Crude fiber	40.9 ± 1.24	41.1 ± 1.82	40.9 ± 0.66	39.5 ± 0.87
Nitrogen free extract	31.5 ± 0.43	32.2 ± 0.37	32.1 ± 0.53	32.9 ± 0.48
Estimated total digestible nutrients	50.1 ± 0.42	50.6 ± 0.25	50.6 ± 0.33	50.8 ± 0.27

<sup>a</sup> 20 analyses

<sup>b</sup> 19 analyses

of weighbacks between groups, with the exception that crude protein was approximately 1.5 per cent higher in the weighbacks of cows on the 45 per cent ration. Since weighbacks were mostly all hay and some silage, the significance of this difference is not understood. Using these average analyses shown in table 4, the total digestible nutrient intake by cows by weeks was calculated. This was compared with maximum requirements for weight and production as outlined in Morrison's tables (8). The results of this study are shown in table 5. The cows, when fed the control ration, gained an average of 25.7 lb.

per 6-wk. period, as compared with 16.8, 18.6 and 8.5 lb. when they had 15, 30 and 45 per cent of their grain replaced with dehydrated grass, respectively. These differences in body weight gains between rations were significant as shown by the analysis of variance. The mean gain per week for the control was significantly higher than the mean gain for the 45 per cent ration. The other mean differences were not significant. The analysis of variance further revealed a

TABLE 5

*Milk produced, changes in body weight, total digestible nutrients consumed, maximum requirements (Morrison, 8) and amount consumed over maximum requirements*

	Rations			
	Amount of grain replaced with dehydrated grass			
	0% <sup>a</sup>	15% <sup>b</sup>	30% <sup>a</sup>	45% <sup>b</sup>
	(lb.)	(lb.)	(lb.)	(lb.)
Av./cow/week	Total digestible nutrients consumed			
	169.5	165.1	164.7	164.9
Av./cow/week	Maximum requirements			
	147.1	142.6	143.0	143.2
Av./cow/week	In excess of maximum requirements			
	22.4 ± 1.15	22.5 ± 1.12	21.7 ± 1.25	21.6 ± 1.45
	Gains in body weight			
Total				
Group 1 <sup>c</sup>	195.0	116.5	45.5	11.0
Group 2	82.5	124.0 <sup>d</sup>	93.5	25.5
Group 3	13.0	91.0	144.0	47.0
Group 4	223.0	12.5	88.5	120.5 <sup>d</sup>
Total	513.5	319.0	371.5	182.0
Av./cow/period	25.7 ± 5.2	16.8 ± 5.3	18.6 ± 2.9	8.5 ± 5.7
Av./cow/week	4.3	2.8	3.1	1.6
	4% fat-corrected milk produced			
Total				
Group 1 <sup>c</sup>	8927.4	8739.8	7225.2	5896.5
Group 2	5888.1	6155.5 <sup>d</sup>	8044.3	6733.2
Group 3	7372.4	6228.2	8588.8	8474.5
Group 4	8743.1	7196.6	5824.6	7379.9 <sup>d</sup>
Total	30931.0	28320.1	29682.9	28484.1
Av./cow/week	257.8	248.4	247.4	249.9

<sup>a</sup> 20 cows

<sup>b</sup> 19 cows

<sup>c</sup> 5 cows/group except where noted

<sup>d</sup> 4 cows

highly significant difference between periods of the trial. The winter of 1948-49 was extremely rigorous during November, December and January. The cows consumed more total digestible nutrients over maximum requirements and gained less during the periods beginning October 16 and December 4 than was the case with the first two periods. Since during this experiment there was a group of cows on each ration each period, this observed difference hardly can be ascribed to the rations. As further shown in table 5, the cows, when fed the 45 per cent grain replacement ration, varied more in weight gains per cow, as evidenced by a comparison of the standard errors. The coefficients of variability

were found to be 90.4, 139.4, 171.0 and 289.6 per cent for the control 15, 30 and 45 per cent rations, respectively. All of these coefficients of variability are high, but period differences attributed to thermal changes also enter in, as was shown earlier from the analysis of variance. A comparison of weight gains with intake of total digestible nutrients above minimum requirements showed negative correlations of  $-0.254$ ,  $-0.367$ ,  $-0.183$  and  $-0.315$  for the control 15, 30 and 45 per cent rations, respectively. These correlations are unusual, but true evaluation of thermal stress is yet to be determined. It is known that animals show increased appetite during cold weather, increased thyroxin output and possibly other physiological adaptations, as outlined by Lee and Phillips (7). The least weight gain and the greatest feed intake over minimum requirements occurred during the October 16 to December 4 period, which coincides with the rather sudden change in thermal conditions which occurred during the fall of 1948.

The production of 4 per cent fat-corrected milk also is shown in table 5 by groups and by rations. Cows on the control ration produced an average of 36.8 lb. of 4 per cent fat-corrected milk daily, as compared with 35.5, 35.3 and 35.7 lb. for the 15, 30 and 45 per cent rations, respectively. The superiority of the control ration for milk production, as compared with the other rations, was further shown by analysis of variance to be statistically significant. The lowered production obtained from the cows receiving dehydrated grass cannot be attributed to the consumption of insufficient total digestible nutrients, since the average daily consumption for these groups was from 3.1 to 3.2 lb. higher than Morrison's (8) maximum standards, as compared with 3.2 lb. daily excess for the cows when on the control ration.

While the total digestible nutrient value of the dehydrated grass is relatively high, thus causing little difference in total digestible nutrient consumption whether on the control, 15, 30 or 45 per cent rations, the crude fiber content of this material was 21.4 per cent, compared with 9.3 for grain. The heat increment necessary to digest the dehydrated grass must be proportionately greater than that of the concentrate mix, thus lowering the amount of energy available for productive purposes, *i.e.*, milk production and deposition of body fat.

Furthermore, the crude fiber content of the grain ration was increased noticeably when dehydrated grass replaced part of the grain. The crude fiber content of the grain ration was 9.3 per cent, as compared with 11.1, 12.9 and 14.8 per cent when 15, 30 and 45 per cent, respectively, of the grain ration was replaced by dehydrated grass. This additional crude fiber may have served to depress somewhat the digestibility of other ration constituents, as indicated by Swift *et al.* (11).

The reduced net energy and increased crude fiber concepts do not seem to explain why there was little difference in the production of 4 per cent fat-corrected milk between the three groups which had a portion of their grain replaced with dehydrated grass, regardless of whether the replacement was a 15, 30 or 45 per cent level. However, as previously pointed out, the cows on the 45 per cent ration gained less. Furthermore, average daily hay consumption varied little regardless of ration, since cows while on the control ration consumed 16.6



lb. of hay daily, as compared with 16.5 lb. for each of the grain replacement rations.

The physical state of the dehydrated grass was undesirable and may be a contributing factor. Several cows showed impaired rumination when 30 per cent of their grain was replaced with dehydrated grass. Three cows were treated for rumen atony while on the 45 per cent ration. Also, additional trouble was experienced from anorexia in varying degrees of severity. These troubles were not evident when these same cows were on the control and 15 per cent rations. These observations are in agreement with those of Cole and Mead (3) and Watson and Ferguson (14). Of course, cows off feed lost weight and dropped off sharply in milk production. Upon recovery, milk flow increased again, but seldom to levels observed prior to digestive disturbances. The rate of lactation decline was least when the cows were on the control ration and greatest when 45 per cent of the grain ration was replaced with dehydrated grass. These differences are clearly shown in figure 1. While the rate of lactation decline in-

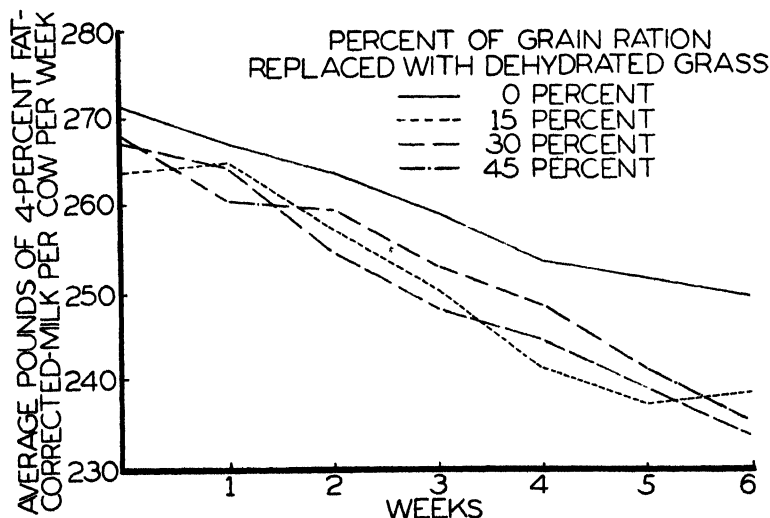


Fig. 1. Average lactation decline by rations for 6-wk. experimental periods.

creased during the fourth, fifth and sixth weeks for the 30 per cent and 45 per cent rations, as compared with the controls, this rate only approached significance ( $t_{.05} = \pm 17.58$  lb.; mean difference between 30 per cent ration and control was 16.0 lb.).

The amount of total digestible nutrients required for maintenance and the production of 100 lb. of 4 per cent fat-corrected milk was 59.9, 62.5, 62.2 and 63.7 lb. for the control, 15 per cent, 30 per cent and 45 per cent rations, respectively. These calculations were made by deducting 3.53 lb. of total digestible nutrients per pound of body gain according to data reported by Knott (6). This does not take in account those instances where cows lost weight during a

portion of, and in some cases during all of, an experimental period. Technically this might alter the results shown above, but it is not considered good dairy cattle management to have cows in mid-lactation lose weight. For practical purposes, the above levels of efficiency would be of importance. Direct comparison of this experiment with other published experiments is not feasible, since the dehydrated grass was a high quality commercial product lower in protein and showed less total digestible nutrients (66.8 per cent digestible) than the materials used in earlier experiments (2, 5, 9, 12, 14). Furthermore, the experimental periods in this trial were 6 wk. in length as compared with 2 wk. for Watson (12) and Knott *et al.* (5), 5 wk. for Watson and Ferguson (14) and 3 wk. for Camburn (2). Newlander (9) replaced all the concentrates with dehydrated grass for 6 wk. and observed a 9.9 per cent decline in milk yield as compared with controls. The average production of cows in this experiment was slightly greater than 35 lb. of 4 per cent fat-corrected milk for the entire 28 wk. experimental period. Several cows were milking in excess of 60 lb. of 4 per cent fat-corrected milk at the start of the experiment.

#### SUMMARY

Twenty high producing dairy cows were divided into four experimental groups consisting of three Holsteins, one Jersey and one Guernsey per group. Three rates of grain replacement (15, 30 and 45 per cent) with a commercially dehydrated grass-legume mixture were studied a total of 28 wk., using 6-wk. experimental periods and 1 wk. for transition for each group of cows for each ration. The following results were observed:

(a) Cows, when fed the control ration, gained an average of 25.7 lb. in body weight per cow per period, as compared with 16.8, 18.6 and 8.5 lb. when they had 15, 30 and 45 per cent, respectively, of their grain replaced with dehydrated grass.

(b) A digestion trial with three sheep showed that the commercially dehydrated material used contained 66.8 per cent total digestible nutrients on an air-dry basis.

(c) Cows fed the control ration produced an average of 36.8 lb. of 4 per cent fat-corrected milk daily, compared with 35.5, 35.3 and 35.7 lb when they had 15, 30 and 45 per cent of their ration replaced with dehydrated grass, respectively. This difference in production in favor of the control ration was statistically significant.

(d) The finely ground physical state of the dehydrated grass-legume mixture was undesirable, since cows having 30 and 45 per cent of their grain replaced showed varying degrees of rumen atony and anorexia. This condition was not observed when the cows were on the control and 15 per cent rations.

(e) Cows receiving no dehydrated grass required less total digestible nutrients for maintenance and production of 100 lb. of 4 per cent fat-corrected milk than when they received dehydrated grass.

(f) The rate of decline in milk yield was less rapid when the cows were fed the control ration, but these mean differences only approached significance.

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# THE EFFECT OF VITAMIN A FROM PRENATAL STORAGE AND FROM INGESTION OF COLOSTRUM ON THE NEONATAL CALF<sup>1</sup>

L. NEZVESKY,<sup>2</sup> H. D. EATON, R. E. JOHNSON, L. D. MATTERSON,  
C. I. BLISS AND A. A. SPIELMAN

*Animal Industries Department, Storrs Agricultural Experiment Station,  
University of Connecticut, Storrs*

The effect of prenatal nutrition on the subsequent performance of the newborn recently has received considerable attention. Both the prenatal storage and colostral content of vitamin A can be increased in the ruminant by dietary means (10, 13, 16, 18, 20, 21). These two sources of vitamin A have been demonstrated in independent work to be available to the neonatal calf (4, 7). In combination, they have been held to be responsible for increases in the levels of plasma vitamin A of neonatal calves and lambs and in superior performance of neonatal Holstein calves, as indicated by greater weight gains and lower incidence of scours (3, 17). With one exception, Fountaine *et al.* (10), the increases followed the prepartum feeding of supplementary vitamin A *per se*. The number of days of feeding the prepartum supplement has varied widely among workers and in experiments by the same worker. The relative contributions of vitamin A to the neonatal calf from prenatal storage and from colostrum were not determined.

Since vitamin A is relatively expensive (the feeding of one million units daily for 30 days prepartum would cost approximately three dollars per cow at present prices), experiments are needed to determine the minimum level and the best feeding schedule for prepartum supplements of vitamin A. However, to establish the optimum performance of the neonatal calf, information is needed as to the relative contribution of vitamin A from prenatal storage and from colostrum. This was the objective of the present study, which has been based upon measurements of total blood hemoglobin, carotene and vitamin A in the plasma and liver, whole blood and plasma ascorbic acid, liveweight, fecal pH and dry matter and incidence and duration of scours.

## EXPERIMENTAL

**Animals.** Twenty-eight cows of the Ayrshire, Guernsey, Holstein and Jersey breeds which had calved in the University of Connecticut herd from November, 1948, through May, 1949, were used in the experiment. One half of these cows received a basal ration for 8 wk. prior to the calculated parturition date, and

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<sup>2</sup> Present address: College of Veterinary Medicine, Cornell University, Ithaca, New York.

the other half received the same basal ration plus one million U.S.P. units of vitamin A daily for 30 days prior to the calculated parturition date. The levels of feeding and the composition of the feeds and supplementary vitamin A<sup>3</sup> are essentially as described previously (5).

The calves born to these cows were not allowed to nurse but were removed to individual pens in the calf barn. There they received colostrum from frozen pooled colostrum banks. The allotment of calves to dietary groups was as follows:

Dams	Colostrum	
	From Basal dams	From basal + vitamin A dams
Basal dams	Group I	Group II
Basal + vitamin A dams	Group III	Group IV

Four separate colostrum banks of basal colostrum and a similar number of basal plus vitamin A colostrum were used and represented the first two milkings after parturition. The first bank was from cows fed rations identical to those in this experiment and was made up immediately prior to the birth of the first calf on this experiment. The second and following banks were made up from cows on the experiment, as the prospective need for additional colostrum became evident, in order to have a supply of colostrum on hand at all times. In the basal colostrum banks, the carotene averaged 278  $\gamma$  per cent, ranging from 249 to 309  $\gamma$  per cent, and the vitamin A, 273  $\gamma$  per cent with a range of 166 to 347  $\gamma$  per cent. Similar values for the basal plus vitamin A banks were carotene, 216  $\gamma$  per cent with a range of 174 to 309 and vitamin A 706  $\gamma$  per cent with a range of 491 to 1217.

The newborn calves received four feedings of colostrum in the first 2 days at the rate per feeding of 3.75 lb. to Ayrshires and Holsteins and 3 lb. to Guernseys and Jerseys. They then were fed herd milk, starter and U. S. no. 2 clover-timothy mixed hay according to the Cornell drycalf starter method (19). Both colostrum and milk were fed in nipple pails. Water was allowed *ad lib.* after the first week. The temperature in the calf barn was maintained at a minimum of 16° C. by radiant heat from the ceiling.

*Samples, observations and analyses.* Venous blood samples were collected immediately after birth, before the second, third, fifth, seventh and tenth feedings of colostrum or herd milk and at 7, 10, 14, 21 and 28 days of age. Total hemoglobin, plasma carotene and vitamin A, and whole blood and plasma ascorbic acid were determined with an Evelyn photoelectric macro-colorimeter by the methods of Evelyn and Malloy (8), Kimble (12), and Roe and Kuether (14), respectively. Calves were weighed at birth and at 4-day intervals thereafter to 28 days of age. Daily fecal samples were obtained until the calf was 15 days of age and, in addition, at 18, 21 and 28 days, by rotating a clinical thermometer

<sup>3</sup> The vitamin A supplement, shark liver oil containing 25 per cent by weight of crude soybean lecithin, was generously supplied by the Nopco Chemical Co., Harrison, N. J.

in the anus of the calf. Fecal pH was determined by the method of Shoskes (15) and dry matter by oven-drying at 100° C. for 24 hr. Four calves each from groups I and III, three from group IV and two from group II were slaughtered at the end of the 28-day experimental period and liver carotene and vitamin A determined by the method of Davies (1).

Standard statistical procedures as outlined by Davies (2) and Fisher and Yates (9) were used to test for differences between treatments. In addition, the liveweight data were analyzed according to the methods proposed by Wishart (23).

#### RESULTS AND DISCUSSION

*Hemoglobin.* Total hemoglobin did not vary significantly between treatments, but decreased with age. Since this trend was in accord with the report of Wise *et al.* (22), the data need not be cited.

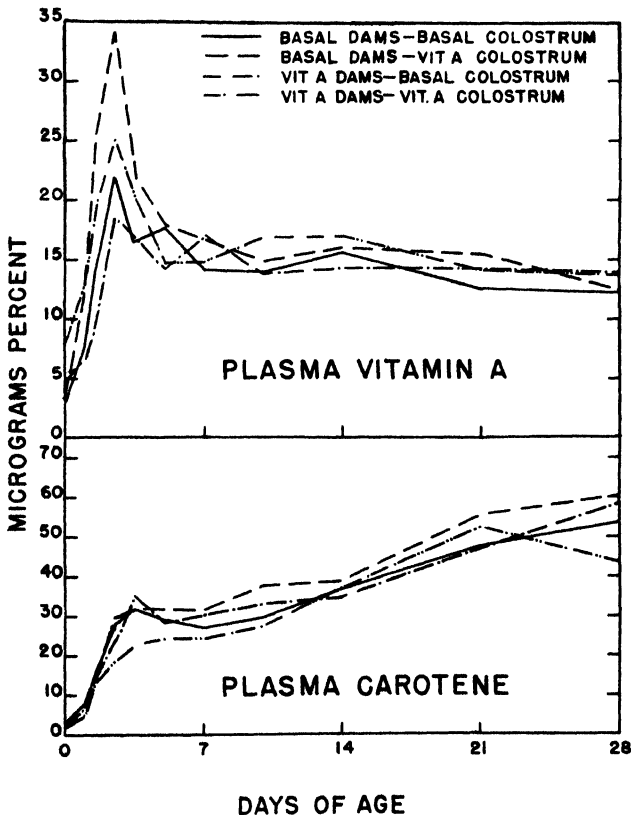


FIG. 1. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the blood plasma levels of carotene and vitamin A in the young calf.

*Carotene and vitamin A.* The levels of plasma carotene (fig. 1) were not affected significantly by treatment. The vitamin A content of the plasma col-

lected for the first 5 days after birth was significantly higher ( $P < 0.001$ ) in both groups of calves fed colostrum from dams receiving prepartum supplements of vitamin A. In later samples from the 7th to the 28th day the difference was in the same direction, though not significant. The plasma level of vitamin A at birth was significantly higher ( $P < 0.01$ ) in calves from dams fed prepartum supplements of vitamin A than that in calves from dams on the basal ration alone.

The liver storage of carotene at 28 days of age (fig. 2) was decreased, but

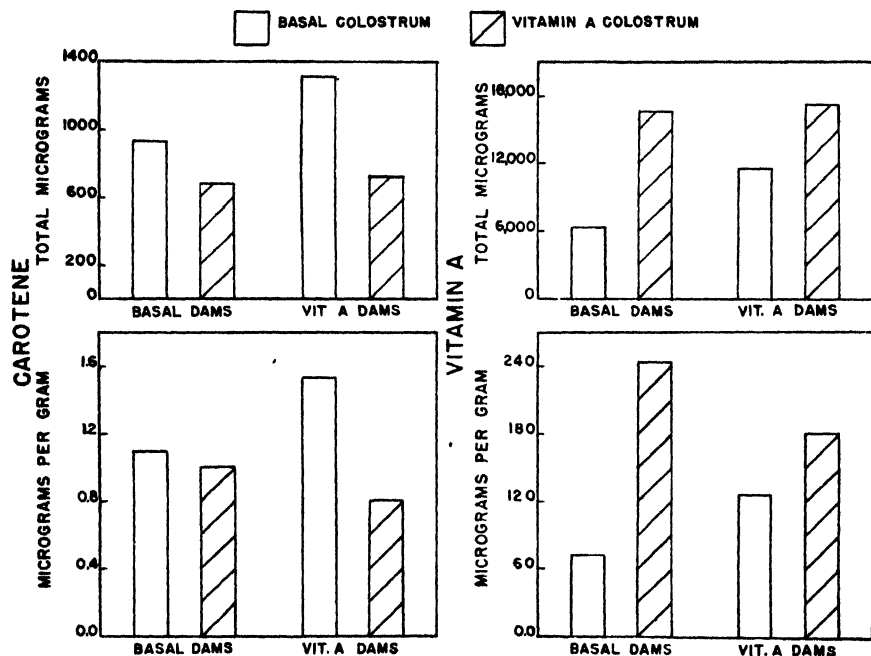


Fig. 2. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the geometric mean content of carotene and vitamin A in the liver of calves at 28 d. of age.

not significantly, by the feeding of colostrum from dams receiving prepartum supplements of vitamin A, but practically unaffected by prenatal storage. The liver storage of vitamin A at 28 days of age was increased markedly ( $P < 0.01$  per gram of liver and  $P < 0.05$  for total liver storage) by the feeding of colostrum from dams fed the supplementary vitamin A. Although the additive influence of prenatal storage on the vitamin A content of the liver was not of statistical significance, an analysis of the liver stores at 28 days in the two groups of calves receiving the basal colostrum showed a nearly significant increase due to the influence of the prepartal supplements of vitamin A. Prepartum supplements of vitamin A increased the level of vitamin A in the neonatal calf, primarily through the colostrum. High levels of vitamin A possibly may be pro-

duced by feeding vitamin A for shorter periods than was the case in this work or in similar studies as reviewed by Parrish *et al.* (13).

*Ascorbic acid.* Neither whole blood nor plasma ascorbic acid (fig. 3) was affected significantly by treatment. The ascorbic acid levels decreased markedly immediately after birth and then continued to fall at a slower rate. These results are essentially in agreement with those of Hibbs and Krauss (11), both

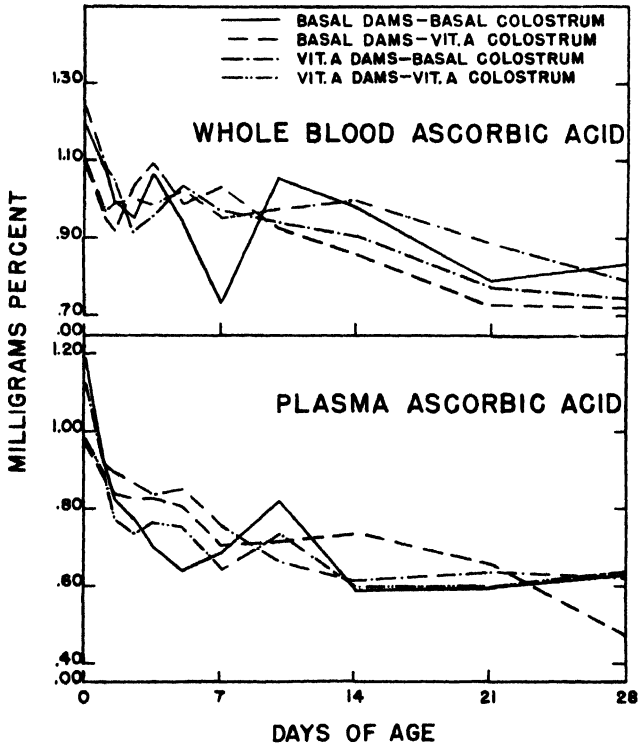


FIG. 3. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the whole blood and plasma ascorbic acid levels in the young calf.

in trend and in the lack of correlation between the plasma and liver level of vitamin A and plasma ascorbic acid in the neonatal dairy calf.

An analysis of all observations for whole blood ascorbic acid and for plasma ascorbic acid showed significant differences ( $P < 0.001$ ) between calves and between ages; however, the level of significance for the plasma ascorbic acid, as indicated by the  $F$ -value, was greater than that for whole blood. In contrast, calculated cellular ascorbic acid, uncorrected for cellular volume, did not show significant differences between calves and gave a lower level of significance ( $P < 0.01$ ) between ages. Therefore, plasma ascorbic acid is a more sensitive criterion of the ascorbic acid level of blood because of the relative stable cellular ascorbic acid content.



**Liveweight.** An analysis of the changes in liveweight (fig. 4), in terms of either total gain or rate of increase (23), with or without adjustment for weight at birth, revealed no significant differences between treatments. Since the number of calves was small and represented four breeds, one could expect only wide differences to be detectable.

**Fecal pH and dry matter and incidence of scours.** Neither fecal pH nor fecal dry matter (fig. 5) showed a significant difference between treatments, but both varied with increasing age. The cause of these changes has yet to be determined.

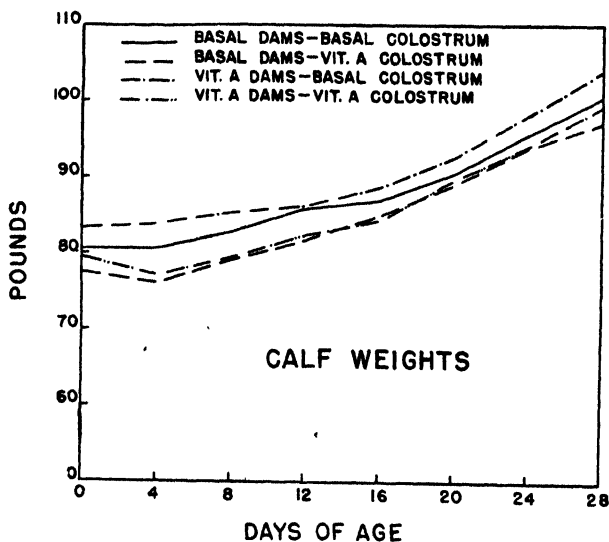


FIG. 4. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the liveweight changes in the young dairy calf.

Five cases of relatively mild scours were observed, but, contrary to expectation, they were accompanied by no appreciable change in the content of fecal dry matter or pH. No group had more than two cases and none showed any relation to vitamin A treatment.

#### SUMMARY

The effect of vitamin A from prenatal storage and from ingestion of colostrum was studied from birth to 28 days of age in 28 dairy calves. One half of these calves were from dams receiving only a basal ration and one half were from dams receiving the same basal ration plus one million U.S.P. units of vitamin A daily for 30 days prior to the calculated date of parturition. At birth the calves from each of the maternal dietary groupings were subdivided into two groups, one receiving colostrum from dams fed only the basal ration and the other receiving colostrum from dams fed the basal ration plus the supplementary vitamin A.

The data indicate that colostrum significantly increased the plasma vitamin

A from birth to 5 days of age and liver vitamin A at 28 days of age. Prenatal storage elevated the blood plasma vitamin A level at birth significantly and contributed to greater liver storage of vitamin A at 28 days of age. The

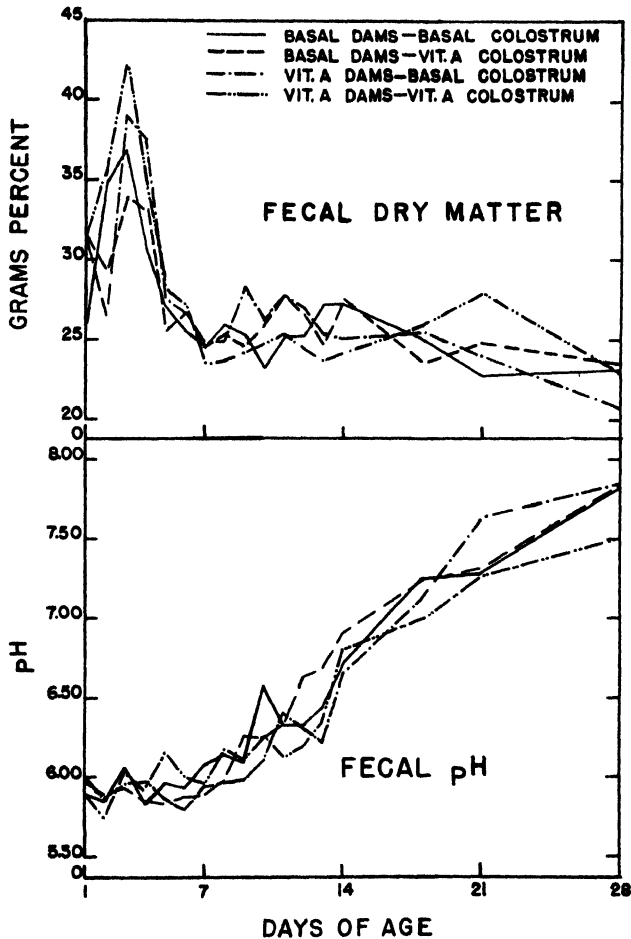


FIG. 5. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the fecal pH and dry matter in the young dairy calf.

other criteria measured, hemoglobin, whole blood and plasma ascorbic acid, liveweight, fecal pH and dry matter and incidence of scours, were not affected significantly by treatment.

#### ACKNOWLEDGMENTS

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# STUDIES OF HEATED MILK. I. FORMATION OF 5-HYDROXYMETHYL-2-FURFURAL<sup>1</sup>

STUART PATTON

*Department of Dairy Husbandry, Pennsylvania Agricultural Experiment Station,  
State College, Pa.*

Some recent research has focused interest on furan compounds and their possible relation to the so-called browning reaction. Singh *et al.* (7) have shown that the darkening of sugar solutions is related to the formation of hydroxymethylfurfural and levulinic acid. Patton and Josephson (5) have isolated furfuryl alcohol from skim milk which had undergone browning as a result of heat treatment. The compound maltol, an isomer of hydroxymethylfurfural, also has been isolated from heated skim milk (4). The importance of furan compounds has been firmly established in the browning of apricot concentrates (1).

Fundamental investigations of the browning reaction by Wolfson *et al.* (8) have revealed that hydroxymethylfurfural is formed by heating aqueous systems of glucose and glycine. These workers also report that hydroxymethylfurfural, in sufficient concentration, forms deeply colored products with glycine and at a high rate of speed.

It is well known that certain dairy products undergo browning rather readily as a result of heat treatment, storage at conducive temperatures or both. Of the various milk constituents, lactose and casein are known to be intimately concerned in the color change (2).

These interesting observations from the literature have suggested the possibility that hydroxymethylfurfural may be a concomitant of browning in heated milk. The present paper reports a fundamental study of this point.

## EXPERIMENTAL

*Preparation of pure hydroxymethylfurfural.* In order to obtain known derivatives of hydroxymethylfurfural, the pure compound was prepared by the method of Middendorp (3) with slight modifications. Sucrose (200 g.) and oxalic acid hydrate (1.4 g.) were dissolved in 600 ml. of water. The solution was heated in an autoclave at 127° C. for 2.5 hr. After cooling to room temperature, the reaction mixture was neutralized with CaCO<sub>3</sub> and then gently agitated with 10 g. basic lead acetate for 1 hr. Suspended material was removed from the mixture by centrifuging. The clear supernatant containing the hydroxymethylfurfural was extracted with ethyl acetate for 10 hr. in a continuous extraction unit. The ethyl acetate extract was dried, the solvent removed and the crude hydroxymethylfurfural purified by distillation under high vacuum; yield 14 g. m.p. 32–33° C.

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The following derivatives, with melting points as indicated, were prepared from the purified hydroxymethylfurfural: semi-carbazone (194–195° C.), phenylhydrazone (140–141° C.) and 5-hydroxymethyl-2-furoic acid (165–166° C.). The melting points of these derivatives were in accordance with values reported for them in the literature (6).

*The interaction of lactose and glycine.* In view of the findings by Wolfrom *et al.* (8) that hydroxymethylfurfural is formed by the heating of aqueous glucose-glycine systems, it was considered of interest to study the character of this reaction as applied to lactose. Glycine, having amino and carboxyl groups, should present a simplified version of similar reactive groupings in a protein particle.

$\beta$ -Lactose (C.P., 150 g.) and glycine (30 g.) were dissolved in 820 ml. of water. This solution (pH 7.0) was autoclaved for 2.5 hr. at 127° C., after which it was cooled to room temperature, neutralized with a 10 per cent suspension of  $\text{Ca}(\text{OH})_2$  and centrifuged. The clear, dark-brown supernatant was extracted with ether for 24 hr. in a continuous extraction unit. The ether extract was dried and, after evaporation of the solvent, a small quantity (0.4 g.) of light brown oil remained. This oil, upon distillation under high vacuum ( $< 0.5$  mm.), proved to be fairly pure hydroxymethylfurfural. The distillate solidified readily in the cold and, on subsequent warming, melted at 32–33° C. It had a bitter, acrid taste and gave an intense purple color in the Molisch test. The semicarbazone and phenylhydrazone derivatives were prepared readily from it and found to melt at 194–195° and 140–141° C., respectively. These derivatives showed no melting point depression on admixture with authentic samples of like melting point.

A similar experiment, in which the only variation was to increase the time of autoclaving to 5 hr., yielded 1.28 g. of crude hydroxymethylfurfural. Small quantities of maltol were formed in these lactose-glycine systems (4), but no furfuryl alcohol could be detected.

*Carbohydrate-casein systems.* With the formation of hydroxymethylfurfural in heated lactose-glycine systems established, it was decided to ascertain the effect of replacing glycine with casein on this reaction. A sodium caseinate sol was prepared as follows: Raw skim milk was coagulated by the addition of 1 *N* HCl. After the curd had settled, the whey was drained and the curd then washed four times, under gentle agitation, with volumes of water equivalent to the whey removed. Resuspension of the acid casein was accomplished by taking it up in water, adjusting the pH to 6.7 with *N*/3 NaOH and agitating the sol for several hours at 20–25° C. The few gross particles remaining were removed by filtration of the sol through fine filter cloth. The total solids content of the sol was determined to be 6.65 per cent.

Lactose (150 g.) was dissolved in 850 ml. of the casein sol and the mixture autoclaved for 2.5 hr. at 127° C. This mixture, following autoclaving, was neutralized, clarified and extracted in the same fashion as the lactose-glycine system except that the heat-coagulated casein curd was pressed in an hydraulic press to obtain the maximum amount of "whey" for ether extraction. An extract

residue of 0.56 g. was obtained after removal of solvent. Vacuum distillation of this residue produced three fractions, the principle constituents of which were 270 mg. of furfuryl alcohol, 70 mg. of maltol (by sublimation), and 160 mg. of hydroxymethylfurfural.

The identification of furfuryl alcohol and maltol was accomplished by a study of their physical and chemical properties, the preparation of suitable derivatives and the performance of appropriate melting and mixed melting point determinations. These procedures have been described previously in detail (4, 5) and were attended meticulously in all experiments reported in this paper. Hydroxymethylfurfural was identified in the manner described for this compound from the lactose-glycine system. This method also was used in subsequent experiments reported herein.

Two additional systems were prepared with the casein sol. In these, lactose was replaced with equal weights of D-glucose and D-galactose (both C.P.). Autoclaving, refining, extraction, etc. of these systems were the same as for that of lactose. No detectable quantities of either furfuryl alcohol or maltol were found in the extract residues obtained in these experiments. The crude residues yielded 0.62 g. of hydroxymethylfurfural for the galactose system and 0.61 g. for that of glucose. These three experiments concerning lactose, glucose and galactose were repeated utilizing a 7.5 per cent aqueous suspension of commercial casein (Arthur H. Thomas Co.) in place of the casein sol. The results were very similar in all respects to those obtained when the casein sol was employed.

*Heated skim milk.* In these experiments, condensed skim milk (30 per cent total solids) was used. The use of this product appeared to have several advantages over using plain skim milk. The reactants in the condensed skim milk are more concentrated, thus less milk need be handled. Further, it was found that better yields of the end-products are obtained when condensed skim milk is used, although qualitative tests showed that the same compounds are produced by heating plain skim milk. The use of whole milk was avoided, since milk fat would have complicated the extraction and purification procedures considerably. It seems unlikely that the presence of milk fat would have had any great bearing on the matter under investigation.

Several very similar experiments were conducted, of which the following is representative. Six liters of condensed skim milk were autoclaved for 2.5 hr. at 127° C. and then cooled to room temperature. The whey (3600 ml.), obtained by decantation from and expression of the heat coagulated curd, was neutralized, centrifuged and extracted with ether as described for the simplified systems. The solvent was evaporated, the final trace under vacuum, and the extract residue (3.0 g.) washed with two 15-ml. portions of petroleum ether to remove lipid material (0.50 g.). The residue was distilled under high vacuum and the following fractions obtained: Low boiling, unidentified, 0.37 g.; furfuryl alcohol, 1.25 g.; maltol (by sublimation), 0.42 g.; hydroxymethylfurfural, 0.11 g.; non-distilling residue, 0.20 g.

The rather disproportionately large amounts of non-distilling residue obtained in the skim milk extraction experiments as compared with those of the

simplified system created a problem. It appeared that weakly acidic substances in the residue were promoting polymerization which slightly reduced the yield and purity of the hydroxymethylfurfural fraction. However, the semicarbazone and phenylhydrazone derivatives were satisfactorily prepared from it and the chemical and physical properties of the fraction were found to be in good agreement with those of hydroxymethylfurfural.

*Control experiments.* One liter of aqueous, 15 per cent lactose solution was autoclaved at 127° C. for 2.5 hr. As a result of autoclaving, this solution showed little discoloration, whereas, the reaction mixtures, from which hydroxymethylfurfural was isolated, all were appreciably darkened in color by autoclaving. No measurable quantity of hydroxymethylfurfural could be recovered by ether extraction of the heated lactose solution.

The ether extraction procedure for recovering hydroxymethylfurfural from aqueous systems was found to be effective. Three grams of hydroxymethylfurfural, dissolved in 2 l. of water, was 93 per cent recovered by continuous extraction with ether for 24 hr.

#### DISCUSSION

Unfortunately, there are no suitable quantitative techniques for measuring hydroxymethylfurfural or furfuryl alcohol in a complex system such as heated milk. Thus, it was necessary to limit the scope of these experiments to compound isolation. As a consequence, no great stress can be placed upon the quantities of compounds isolated, since very small yields were obtained and some losses during isolation were inevitable. Therefore, where quantitative data have been presented, the primary purpose was to show that the compounds were recovered in some quantity in relatively pure form.

These experiments have demonstrated that hydroxymethylfurfural is formed during prolonged heating of concentrated skim milk. The recovery of the compound from heated lactose-glycine and lactose-casein systems has indicated that lactose is the origin. The results of the control experiment have shown that the conversion of lactose to small quantities of hydroxymethylfurfural is facilitated by the presence of glycine, casein or the heat degradation products of casein. Since it was observed that a pure lactose solution did not darken, whereas all the systems forming hydroxymethylfurfural browned readily, under the experimental conditions, it would appear that hydroxymethylfurfural formation is associated with browning in these systems. It is interesting to note that cleavage of the  $\beta$ -linkage of lactose is implied in its conversion to hydroxymethylfurfural. The experiments with glucose and galactose revealed that either hexose portion of the lactose molecule could give rise to the compound.

The compound which is recovered most readily and in highest yield from heated skim milk is furfuryl alcohol. Patton and Josephson (5) have suggested that either lactose or ascorbic acid might serve as the origin of furfuryl alcohol in heated skim milk. These experiments effectively preclude ascorbic acid as a possible origin, since yields of furfuryl alcohol exceeding that theoretically possible from ascorbic acid were obtained from heated skim milk. In addition, furfuryl alcohol was recovered from a lactose-casein system containing no ascorbic



acid. The mechanism of furfuryl alcohol formation from lactose is presently under investigation and will be the subject of a subsequent paper.

#### SUMMARY

Hydroxymethylfurfural has been identified as one of the compounds formed during prolonged heat treatment of concentrated skim milk. From studies of simplified systems, it has been shown that this compound is formed from lactose. It further was demonstrated that either the glucose or galactose portion of the lactose molecule may serve as the origin of hydroxymethylfurfural. Glycine, casein or the heat degradation products of casein were found essential in the conversion of lactose to hydroxymethylfurfural. No measurable quantities of hydroxymethylfurfural were formed by heating lactose solutions alone under the experimental conditions employed. The formation of hydroxymethylfurfural appeared to be directly associated with heat-induced browning in these experiments.

In addition to hydroxymethylfurfural, furfuryl alcohol and maltol were formed during the heating of lactose-casein systems. Previous findings (4, 5) with regard to furfuryl alcohol and maltol formation in heated skim milk were confirmed.

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PARTURIENT PARESIS. VI. SOME CHANGES IN THE URINARY EXCRETION OF CERTAIN CONSTITUENTS AT PARTURITION AND THEIR POSSIBLE ASSOCIATION WITH CHANGES IN THE BLOOD PICTURE<sup>1, 2, 3</sup>

T. H. BLOSSER

*Department of Dairy Husbandry, State College of Washington, Pullman*

AND

VEARL R. SMITH

*Department of Dairy Husbandry, University of Wisconsin, Madison*

Since the time when Little and Wright (16) first showed a marked diminution of blood calcium in cows with parturient paresis, many theories have been advanced to explain the blood changes which occur. Unfortunately, not many of the theories are supported by experimental evidence.

It is well known that certain hormones can alter the renal threshold for various blood constituents. For example, in hyperparathyroidism, according to Grollman (10), there is a lowered renal threshold for phosphorus. As a result, the blood phosphorus is lowered, and the urinary excretion of phosphorus is considerably increased. The parathyroids also exert some influence on the urinary excretion of calcium.

Shorr *et al.* (22, 23) demonstrated that certain of the steroidal sex hormones influence the urinary excretion of calcium and citric acid. There was no accompanying change in the blood composition.

In the expulsion of the placenta at the time of parturition, the animal loses the source of considerable hormone production. Following this loss, there undoubtedly is considerable interplay among the endocrine glands in readjusting to the changed conditions following parturition. It is not inconceivable that in this dynamic state of affairs the renal threshold for certain of the blood constituents might be altered or the urinary excretion changed by some other mechanism.

In addition to the above possibility, it also is possible that some sort of kidney disfunction might occur in connection with milk fever. In humans, renal disfunction frequently is associated with diseases occurring during pregnancy and at parturition. In eclampsia, for example, the toxemia responsible for the convulsive state may arise as the result of an improperly functioning kidney (1).

Several investigators (5, 8, 11, 12, 13, 18, 21) have studied the urine of nor-

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<sup>2</sup> Published with the approval of the Directors of Agricultural Experiment Stations of State College of Washington and University of Wisconsin.

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mal and milk-fever cows from a qualitative standpoint. No one has reported quantitative studies made in connection with the urinary excretion of the several constituents known to be involved in milk fever.

Since there is a paucity of information on some of the more basic changes which occur in milk fever and because of the possibility of kidney involvement, studies were initiated to determine quantitatively the urinary excretion of certain substances whose blood level is known to change during the course of the disease.

#### EXPERIMENTAL PROCEDURE

Eight Jersey cows were used in the urinary excretion studies reported herein. Four of the cows were in the University of Wisconsin dairy herd and four were in the herd of the State College of Washington.

All cows in this experiment were handled in a conventional manner at, and following, parturition. With one exception, cows developing milk fever were treated by the injection of calcium gluconate. Cow B63 was treated by the udder inflation method.

Quantitative studies on urinary excretion necessitate collection of all urine excreted over a 24-hr. period. It soon was found that all urine could be collected with a minimum of labor by inducing micturition every 2 hr. according to the method developed by Turner (27).

At the end of a collection day, the 24-hr. specimen was measured, and an aliquot was taken to be used for subsequent chemical analysis. The aliquot was acidified and stored under toluene.

Attempts were made to collect 24-hr. urine specimens 10 and 5 days prepartum, and starting with the third day prepartum through parturition to the third day postpartum. Collections also were made 5 and 10 days postpartum. When cows with parturient paresis were treated by calcium therapy, no subsequent collections were taken during the first 4 days postpartum, because of the confusing effect which calcium gluconate has on the urinary excretion picture.

It is not possible to predict accurately the exact day of parturition. Consequently, the prepartum data do not always conform to the specific standards which were originally set, insofar as collection days are concerned.

Blood samples were drawn daily from the external jugular vein at about the middle of the collection day. With the milk fever cows, blood samples were drawn immediately prior to treatment, even if a sample had been drawn only a few hours previous.

The following methods were used in the analysis of blood: Blood serum calcium, method of Clark and Collip (4); blood serum magnesium, method of Simonsen *et al.* (24); blood plasma inorganic phosphorus, method of Fiske and Subbarow (9).

Urinary constituents were analyzed by the following methods: Calcium, method of Morris *et al.* (19); magnesium, method of Briggs (3); inorganic phosphorus, method of Tisdall (26).

Both blood serum and urinary citric acid determinations were made by the method of Perlman *et al.* (20) in the studies made at the University of Wisconsin.

TABLE 1  
Average levels of certain blood constituents for specific periods of time previous and subsequent to parturition

Days prepartum	Calcium (mg. %)			Magnesium (mg. %)			Citric Acid (mg. %)			Inorganic phosphorus (mg. %)		
	Normal cows	Milk- fever cows	Border- line cows	Normal cows	Milk- fever cows	Border- line cows	Normal cows	Milk- fever cows	Border- line cows	Normal cows	Milk- fever cows	Border- line cows
More than 5 d. ....	10.9	11.4	10.9	2.30	2.43	2.45	4.33	4.93	6.77	5.50	3.12	4.08
5 and 4 d. ....	10.5	10.8	10.2	2.30	2.19	2.36	4.33	5.05	4.52	6.48	4.02	5.38
3 and 2 d. ....	10.4	11.5	10.4	2.19	2.39	2.41	5.31	6.20	5.28	6.61	4.26	5.39
1 d. ....	10.6	10.6	10.6	2.42	2.33	2.50	5.12	9.12	6.61	4.44	3.94	2.40
Day of parturition	10.9	6.0	6.5	2.20	3.08	3.30	3.82	3.65	4.44			
Days postpartum												
1 d. ....	9.2	5.8	7.0	2.45	3.74	3.38	4.70	3.53	2.65	3.96	2.61	3.75
2 and 3 d. ....	10.0	6.8	8.4	2.30	3.25	2.78	5.12	1.74	5.00	4.46	3.43	4.77
4 and 5 d. ....	11.5	10.5	10.2	2.34	1.88	2.02	5.63	4.19	3.30	5.36	4.77	3.44
10 d. ....	11.1	11.0	11.0	2.30	1.96	1.79		6.18	4.78	5.73	5.44	4.19

TABLE 2  
Average 24-hour excretion of certain urinary constituents for specific periods of time previous and subsequent to parturition

Days prepartum	Calcium (g.)			Magnesium (g.)			Citric acid (g.)			Inorganic phosphorus (g.)		
	Normal cows	Milk- fever cows	Border- line cows	Normal cows	Milk- fever cows	Border- line cows	Normal cows	Milk- fever cows	Border- line cows	Normal cows	Milk- fever cows	Border- line cows
More than 5 d. ....	0.157	0.973	0.142	1.851	4.646	2.869	0.421	1.621	0.591	0.0294	0.0266	0.0197
5 and 4 d. ....	0.410	0.638	0.394	1.657	3.580	3.347	0.605	1.264	1.537	0.0176	0.0345	0.0339
3 and 2 d. ....	0.333	0.139	0.206	1.391	2.822	2.961	0.700	2.959	0.990	0.0447	0.0231	0.0317
1 d. ....	0.083	0.334	0.096	1.629	1.815	2.856	0.372	3.199	0.946	0.0514	0.0252	0.0252
Day of parturition	0.432	0.091	0.074	1.820	1.611	1.474	1.257	0.908	0.795	0.0778	0.0239	0.0534
Days postpartum												
1 d. ....	0.082	0.114	0.298	2.782	1.670	2.569	0.806	0.314	0.965	0.0622	0.0319	0.3416
2 and 3 d. ....	0.256	0.232	0.232	2.991	2.667	2.667	0.869	0.915	0.915	0.557	3.5681	0.2533
4 and 5 d. ....	0.427	0.130	0.251	2.896	1.959	1.404	0.754	0.585	0.700	0.0305	0.8033	0.2533
10 d. ....	0.191	0.239	0.336	3.328	2.755	2.393	1.355	0.901	1.188	0.0698	0.0734	0.6348

sin and by the method of Taussky and Shorr<sup>4</sup> (25) in studies made at the State College of Washington. The latter method was somewhat more sensitive and reproducible, but over-all results using the two methods were essentially the same.

#### RESULTS AND DISCUSSION

Of the eight Jersey cows which were studied, four developed definite symptoms of parturient paresis and subsequently were treated for the disease. Two of the remaining four cows did not show symptoms of milk fever and can be considered as having normal uncomplicated parturitions. The remaining two cows did not "go down" with milk fever and were not treated, but they did appear slightly unsteady for a short period of time following parturition. The blood composition of these two cows paralleled much more closely the typical blood composition found in a milk-fever cow than that found in a normal cow at parturition. Though the latter two cows cannot be considered as having milk fever, their blood composition was so much different from that of a normal cow that to include them in normal cow averages would confuse the data. Therefore, they are treated as a separate group and are referred to hereafter as "borderline" cows.

Tables 1 and 2 present average values obtained of blood and urinary constituents of the three groups prior and subsequent to parturition.

*The blood picture at parturition.* Levels of the various blood constituents which were studied in this experiment were about the same as those previously reported in the literature for cows at parturition (2, 6, 7, 14).

The blood serum calcium dropped about 10 per cent (10 to 9 mg. per cent) in normally freshening cows and about 40 per cent (10 mg. per cent to 6 mg. per cent) in cows which developed milk fever. Blood calcium levels dropped almost as much in borderline cows as in milk-fever cows.

The blood serum magnesium in cows freshening normally did not change from prepartal levels as a result of parturition. Both the milk-fever and the borderline cows exhibited a marked increase in serum magnesium levels on the day of parturition. These values rose to still higher levels 1 day postpartum, but by 4 days postpartum the values had returned to prepartal levels.

The average drop in blood plasma inorganic phosphorus was to 4.44, 2.40 and 2.06 mg. per cent at parturition in the normal, borderline and milk-fever cows, respectively. The magnitude of the drop was not greatly different in the three groups between 2 days before parturition and day of parturition, since the normally freshening group had a considerably higher level of blood plasma phosphorus 2 days before parturition than did either of the other groups.

Levels of blood serum citric acid at calving followed the same pattern as that described by Blosser and Smith (2). There was a drop in the serum citric acid at parturition in all groups, but the milk-fever and borderline cows exhibited a greater drop than the normally-calving cows.

<sup>4</sup> n-Heptane used in the citric acid analyses by the method of Taussky and Shorr (25) was generously supplied by the Phillips Petroleum Co., Bartlesville, Oklahoma.

*Excretion studies at parturition—urinary calcium.* The 24-hr. urinary excretion of calcium did not seem to vary markedly in any of the three groups. No one group of the three consistently excreted excessively larger or smaller amounts of calcium via urinary channels than either of the other two groups. One of the cows, which subsequently developed milk fever, excreted in excess of 3 g. of calcium during the 24-hr. period on the tenth day prepartum. This was more than five times as much urinary calcium as was excreted by the same cow or any other cow, at any other time during the collection period, either pre- or postpartum. It is doubtful whether any significance should be attached to this variation, since it was shown by only one cow on 1 day.

There was not an inverse relation between the urinary excretion of calcium and the blood serum levels. The drop in blood serum calcium at parturition was not accompanied by an excessive excretion of this constituent via urinary channels. Thus an explanation for the dramatic drop in blood serum calcium which occurs in parturient paresis will have to be sought for elsewhere.

*Urinary magnesium.* There were distinct differences in the prepartal urinary excretion of magnesium by the milk-fever and borderline cows on one hand, and the normal cows on the other hand. There is not a sufficient amount of data to compare the three groups more than 5 days prepartum.

The cows which subsequently developed milk fever excreted larger amounts of magnesium via the urinary route between 16 and 3 days prepartum than did any of the cows of either of the other groups at any time, either pre- or postpartum. Daily excretion of magnesium was especially high in the milk-fever cows studied between 15 and 8 days prepartum. The urinary excretion of magnesium was quite constant between 7 and 3 days prepartum, following which there was a considerable drop on the second day prepartum. There was a still further decline in magnesium excretion on the first day prepartum. The average magnesium excretion was essentially the same on the day of parturition for the milk-fever, borderline and normal cows (milk fever, 1.611 g.; borderline, 1.474 g.; normal, 1.820 g.). The borderline group followed essentially the same excretory pattern for urinary magnesium as did the milk fever group, except for the fact that their greatest decline came between 1 day prepartum and the day of parturition.

The normally-freshening cows excreted magnesium at fairly constant levels prepartum, via the urinary route. Between 2 days prepartum and the day of parturition, however, there was a slight increase in urinary excretion of magnesium. This is in contradistinction to the definite decrease which occurred in both the milk-fever and borderline cows during the same time.

There was a close parallel between the normal and borderline groups for the first 2 days postpartum. Both groups showed an increased urinary excretion of magnesium during this time. However, on and following the third day postpartum, while the normal groups continued to excrete 3 g. daily of magnesium, in the borderline cows the magnesium excretion again dropped, and, on the fifth and tenth day postpartum, more closely paralleled magnesium excretion in the cows which had had milk fever than the normal cows. Thus, the magnesium

excretion in both the milk-fever and in the borderline cows was substantially less than in the normal cows on the fifth and tenth days postpartum.

If an inverse relation existed between urinary excretion and the blood levels of any of the constituents studied in this trial, magnesium was that constituent. However, the days on which the major changes occurred in each are not coincident. The greatest drop in urinary magnesium excretion in the milk fever cows occurred between the third and first days prepartum, but the greatest increase in blood serum magnesium occurred between 1 day prepartum and 1 day postpartum. Furthermore, the decline in serum magnesium levels to normal, following parturition, was not accompanied by a substantial increase in urinary excretion of magnesium. In addition, while the serum magnesium levels remained essentially unchanged in normal cows over a period of time extending from 5 days prepartum to 5 days postpartum, the urinary excretion of magnesium nearly doubled during that time.

Calculations show that changes occurring between days in urinary magnesium excretion at the time of parturition could easily account for blood changes in magnesium of the magnitude observed in milk fever. However, in view of the information presented above, and since considerable magnesium also is excreted via fecal channels and in the milk, a complete balance study would be necessary to determine whether or not urinary excretion is chiefly responsible for the blood changes in magnesium at parturition.

*Urinary citric acid.* There were pronounced differences between the milk fever group and the normal and borderline groups in the excretion of citric acid. No significance can be attached to the differences in urinary excretion of citric acid between the groups more than 5 days prepartum, since only a few comparisons are available. However, on the third, second and first days prepartum, there was consistently a greater excretion of citric acid in the cows which subsequently developed milk fever than in either the normal or the borderline cows. This is particularly interesting in view of the increased levels of blood serum citric acid which were demonstrated to occur prepartum in cows subsequently developing milk fever (2).

In the normal and borderline groups, urinary excretion of citric acid was not appreciably different during the first 4 days postpartum than for the 4 days immediately preceding parturition. Only scanty data on postpartum urinary excretion in milk-fever cows are available in this study, since urine collections were not usually made for the first 4 days postpartum if the cow developed milk fever. On the fifth and tenth days postpartum, urinary excretion was essentially the same in all groups.

A rise in the urinary excretion of citric acid was not accompanied by a fall in blood serum citric acid. For the most part there seemed to be a direct relation between urinary excretion and blood serum levels, particularly in the milk fever group. Thus, an increase in urinary excretion of citric acid was accompanied by an increase in blood serum levels in the milk-fever cows.

*Urinary inorganic phosphorus.* Maynard (17) has pointed out that the herbivores excrete very small amounts of phosphorus through urinary channels.

Ninety-four per cent of the urinary phosphorus excreted is in inorganic form.

Very small amounts of phosphorus were excreted in the urine at any time prepartum by any of the groups. The usual amounts excreted during this time were from 0.02 to 0.04 g. daily. There was no change in the urinary excretion of phosphorus postpartum in the normal group, as compared with prepartal excretion in the same group.

There was a very dramatic increase in inorganic phosphorus excretion by both borderline cows on the second day postpartum. These two cows excreted about 5.0 g. on the average for this day. This was more than 100 times as much inorganic phosphorus as was excreted by the same cows at any other time prepartum and about ten times as much inorganic phosphorus as was excreted by the same cows at any other time postpartum.

On the fifth day postpartum, the milk fever group excreted considerably more inorganic phosphorus than at any other time pre- or postpartum, but on the tenth day postpartum, urinary excretion of this constituent had returned to its normal low levels. On the other hand, the borderline group was still excreting substantially larger amounts on the tenth day postpartum than they had excreted at any time prepartum. This amount also was considerably in excess of that excreted by either of the other groups on the same day.

No definite relationship existed throughout the study between blood plasma inorganic phosphorus and the urinary excretion of this constituent. In the borderline group, however, for each increase in the daily urinary excretion of inorganic phosphorus there was a concurrent increase in the blood plasma levels. This relationship held for every day postpartum during which blood samples were taken and urine collections were made.

It is important to note specifically that the trends observed in the preceding paragraphs are based on a relatively small number of cows. The behavior patterns of the various groups, insofar as urinary excretion is concerned, might change somewhat, particularly in excess of 5 days prepartum, if further studies were made. The fact that the blood composition, even with a small number of cows involved in the average, was quite typical of both normal and milk-fever cows and that the urinary excretion data was quite consistent for cows of the different groups, leads one to believe that the urinary excretion data at parturition as established in this study is quite typical of that which would be found if more cows were studied.

#### SUMMARY

Urinary calcium excretion by eight Jersey cows was quite variable at parturition. There was no consistent or appreciable variation between the normal, milk-fever and borderline cows. Neither did a relation exist between calcium losses via urinary channels and changes in the blood calcium.

Urinary magnesium losses were much more extensive between days 16 and 3 prepartum in the cows which developed milk fever than in the normal cows. The borderline cows were intermediate between the other groups in this respect. All three groups were excreting essentially the same amounts of magnesium via the urinary route on the day of parturition. Following parturition, the mag-



nesium excretion by the normal group increased, and by days 5 and 10 postpartum, the normal cows were excreting more urinary magnesium than either the milk-fever or borderline groups. In general, blood serum magnesium seemed to rise in the milk-fever and borderline cows as the urinary excretion of this constituent decreased, but this relation was not consistent throughout the trial.

There was approximately five times as much citric acid excreted in the urine of the cows which subsequently developed milk fever on days 3, 2 and 1 prepartum, as was excreted in the urine of the normal or borderline cows on those days. The high levels of citric acid excretion were accompanied by levels of blood serum citric acid in the milk-fever cows which were higher than normal.

Very small and consistent amounts of inorganic phosphorus were excreted by all of the cows prepartum, with no group differences. In general, the postpartal excretion also was very small. There was no apparent relation between blood plasma levels of inorganic phosphorus and the urinary excretion of this constituent.

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# CHANGES IN MILK PRODUCTION WITH AGE AND MILKING FREQUENCY<sup>1</sup>

JAY L. LUSH AND ROBERT R. SHRODE<sup>2</sup>

*Iowa State College, Ames*

## INTRODUCTION

It is well known that milk production increases with age at an ever-decreasing rate until maximum production is reached at around 6 to 8 yr. Production then declines with advancing age. This makes the regression of production on age distinctly curvilinear but the nature and amount of that curvature does not appear deducible from any general physiological principles. Consequently, age-changes have been estimated empirically from experiments or from non-experimental data thought suitable. Real but minor differences between breeds in these age-changes have been indicated by some evidence. Indeed such differences are to be expected if breeds really differ in their rates of maturity.

The present investigation primarily was undertaken to measure how production changes with age, using a method thought to be nearly free from the effects of concurrent selection. At least, whatever bias is left by this method would be in the direction opposite to the bias in most of the earlier methods. Kendrick (4) appears to have used the same method, at least in part, but did not publish the details of his procedure.

The effect of variation in times milked per day was studied also because the question is important and the data which had been arranged for the primary purpose also seemed suitable for this with only a little extra work.

## THEORETICAL CONSIDERATIONS

The primary purpose of correction factors is to remove phenotypic differences which occurred because the environmental conditions were not uniformly those chosen as standard. The correction factors ought not to remove from the records any differences really caused by things inherent in the cows themselves. However, the inherent and the environmental causes of differences are often confounded, especially in non-experimental data, so that it is difficult or impossible to separate the effects of one cleanly from the effects of the other. For example, if at each age some cows with low records are culled, then the older cows will include a larger fraction of those with inherently high production and a smaller fraction of inherently low producers than are among the cows which make records at the younger ages. If the regression of production on age is computed from the averages of all data available at each age, that curve will not show the effects of age alone but will show those effects combined with whatever effects such culling actually had.

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<sup>2</sup> Now Associate Professor of Genetics, Texas Agricultural and Mechanical College.

An opposite bias is introduced if the average inherent productivity of the dairy population is increasing. In that case, at any given date the averages for the older ages do not yet include records from the cows born in the most recent years when the average productivity of the population had become higher. In this way the effects of any genetic time-trends in inherent productivity may be confounded with the effects of age.

As another example of confounding, consider estimating the effects of milking three times per day instead of twice a day by merely comparing the average production of all  $3 \times$  and all  $2 \times$  cows. In some of the herds the policy may have been to milk thrice a day those cows which start out to produce well, but to milk only twice a day those cows whose initial production seems too low to make the extra milking worth while. To the extent that such a policy is followed, the differences in production of the  $2 \times$  and  $3 \times$  cows will be, in part, the causes of whether the cows were milked twice or thrice, instead of the other way around.

Where cause and effect can be intertangled so intricately, it is difficult to know whether one has measured the true size of each separate effect, especially on non-experimental data collected from the field. Yet experiments designed expressly to prevent such confounding would need to be on an expensively large scale, if they are to yield accurate results. This is so because the variability between cows is large and the repeatability of a cow's production from one lactation to another is moderately low, even when every effort to repeat the environmental conditions is made.

Correction factors may be called *statistical control* which is used instead of some *physical control* which was not actually achieved. Both kinds of control are intended to remove variations caused by circumstances thought not pertinent to the question being investigated. Physical control is the more expensive—often vastly more so—and frequently is not possible. Statistical control is subject to error from the correction factors being imperfect and, of course, can be applied only to sources of variation which were known. Physical control also is far from perfect, as is well appreciated by anyone who has ever tried to hold conditions constant for a group of cows through two or more successive lactations and then has measured the correlation between their records in one lactation and in another. That correlation should be  $+1.0$  if one really did succeed in maintaining all the pertinent conditions constant. But usually it is only of the order of  $+0.4$  to  $+0.5$  for fat or milk and somewhat higher for test.

When using either physical or statistical control, some knotty questions arise if there are genuine interactions between the inherent differences in the cows and the variations for which control or correction is to be made. For example, if cows differ genuinely in their rate of maturity for milk production, then age correction factors, even though accurate for the average, will over-correct for some individuals and correspondingly will under-correct for others. The difficulty cannot at all be avoided by physical control, i.e., by comparing only records made at the same age. For, even if one knew that cow A would mature at a younger age than cow B, how would one then decide whether to compare them in first lactations, or as 5-yr.-olds, or at some other age? It seems impossible

to determine differences in rate of maturity for individual cows, although that can be done for genetically distinct groups, such as breeds. Another example of interaction would occur if some cows genuinely respond more to an extra milking than do others. Then the individual cows would rank differently when milked twice and when milked thrice a day. Statistical control by using an average correction would not rank them the same as physical control by milking them all the same number of times. Yet one wishing to use the physical control may have difficulty in deciding which milking frequency he should use. The soundest general criterion is to use as standard the same kind of environment he thinks will be used by the customers for whom he is breeding these cattle. But what if he thinks some of his customers will use  $2 \times$  and others will use  $3 \times$  milking? Fortunately for practical use, it appears that these "interactions" are triflingly small in comparison to other uncontrolled causes of variation in the records, although they do occur.

The bias which concurrent selection introduces into age correction factors derived from the averages of all records made at each age (method A) already has been mentioned. The reasons for this bias generally are well understood. Some have thought to escape it by comparing only records made by the same cows at two successive ages (method B), as we have done here. But, when method B is used, concurrent selection introduces a bias in the opposite direction. Since that is not generally so well understood, a comparison of the biases in methods A and B seems appropriate here.

Let  $N$  = number of cows which have a record at a given age;  $k$  = number of these which are kept to make a record at the next age;  $c = N - k$  = number of cows which die or are sold or culled before they make another record;  $K$  = the mean production of the  $k$  cows at the first age;  $K'$  = the mean production of the  $k$  cows at the next age;  $C$  = the mean production of the  $c$  cows at the first age; and  $C'$  = the mean production which the  $c$  cows would have had in the next lactation if they had been kept.

Then the true age change, free of the effects of selection, would have been  $\frac{kK' + cC'}{N} - \frac{kK + cC}{N} = \frac{k}{N}(K' - K) + \frac{c}{N}(C' - C)$ . Under method A, the apparent age change is  $K' - \frac{kK + cC}{N}$ . From this it follows that the bias (true change minus apparent change under method A) is  $\frac{c}{N}(C' - K')$ . Under method B the apparent age change is  $K' - K$ . From this it follows that the bias (true change minus apparent change under method B) is  $\frac{c}{N}[(C' - K') - (C - K)]$ . The difference between the two methods, which is also the difference between their biases, is  $\frac{c}{N}(C - K)$ .

Now if all of the  $c$  were culls for low production and if this culling were done wholly on the individual records of the cows and if the repeatability of individual records is  $t$ , then  $C' - K' = t(C - K)$ . In that case, the bias in method A would be

$\frac{c}{N}t(C-K)$  and the bias in method B would be  $-(1-t)\frac{c}{N}(C-K)$ . From this the ratio of the two biases would be  $-\frac{1-t}{t}$ . The minus sign merely says that the two biases are in opposite directions. If  $t$  is less than 0.5, the bias in method B actually would be larger than that in method A. But culling for low production is not done wholly on individual records and many of the  $c$  are not culls. Basing the culling partly on type or on the records of dam, or sisters or other relatives close enough to have any practical use for predictive value, has the same effect here as making  $t$  larger and  $C-K$  smaller than they would be if the same fraction of the cows were culled wholly on their own records. The presence of non-culls in  $c$  keeps the ratio  $K'/K$  from being so much smaller than  $C'/C$  as it would be if all of the  $c$  were culled because of their own low records.

Further speculation about the relative sizes of the two biases appears fruitless until more is known about the amount and kinds of selection which actually are practiced. Seath's study (8) seems to be the only extensive one yet published in that field. That study merely determined on two groups of DHIA data the size of  $K-C$  at various ages. The size of the bias is affected by  $\frac{c}{N}$  which, with an average productive life of about 4 yr. for dairy cows, would be somewhat larger than 0.25 from 1 yr. to the next. It was around 0.3 in Seath's data. The size of the bias also depends on  $K-C$ , which would be small if most of the  $c$  who go do so for reasons other than their production.  $K-C$  could be fairly large at the ages, if any, when most of  $c$  are culls because of their own low production. Seath found evidence that the individual culling was more intense among the 2-yr.-olds and 3-yr.-olds than at later ages. At the younger ages in his data,  $K-C$  was of the order of 55 to 70 lb. of fat or 1000 to 1600 lb. of milk. Of course, all cows must leave the herd eventually. If more culling is done at the younger ages, less room is left to cull at the older ages.

The present state of knowledge leaves uncertain the size of these biases from concurrent individual selection, but clearly they are in opposite directions in methods A and B. The true age-change therefore should be between the apparent changes yielded by methods A and B, unless still other highly important causes of change in production are closely confounded with age.

#### DATA USED

The data came from the files of the Holstein-Friesian Association of America<sup>3</sup> in 1943 and consisted of 43,573 Herd Improvement Test records from 11,001 cows. Both  $2 \times$  and  $3 \times$  records were included, 20,893 being  $2 \times$  records from 5,374 cows and 22,680 being  $3 \times$  records from 5,627 cows. Each record, coded to the nearest 10 lb., was the total milk yield within 365 days after freshening.<sup>4</sup>

<sup>3</sup> The authors are indebted to H. W. Norton, Jr., for making these data available.

<sup>4</sup> Since 305-day records now are used much more widely than 365-day records and this change seems likely to be permanent, some details about the general applicability of the findings must remain a bit uncertain. Many of the records did end within 305 days, only about 40 per cent extending for a full 365 days. The average length of HIR records, as found in another study, was about 324 days.

The  $2 \times$  and  $3 \times$  records ranged from 3,500 lb. to 25,000 lb. and from 3,600 lb. to 34,000 lb., respectively. The respective means were 11,404 lb. and 13,876 lb. Table 1 shows the average production for the various ages grouped as is customary in the breed association procedure. The age trend in these averages is shown graphically in the upper left corner of figure 3.

TABLE 1  
*Averages of all records at various ages*

Age group	Milk yield	
	2× milking	3× milking
	(lb.)	(lb.)
Jr. 2	9,471	12,318
Sr. 2	10,046	12,578
Jr. 3	10,419	12,700
Sr. 3	11,046	13,373
Jr. 4	11,409	13,732
Sr. 4	11,912	14,167
Jr. 5	12,093	14,493
Sr. 5	12,168	14,491
6 yr.	12,323	14,803
7 yr.	12,444	14,813
8 yr.	12,248	14,844
9 yr.	12,044	14,919
10 yr.	11,830	14,532
11 yr.	12,254	14,396
12 yr.	11,947	14,261
13 yr.	11,531	14,298
14 yr.	12,660	13,918
15 yr.	11,814	14,889

Each record was taken from the files without regard to its magnitude, the only requirement being that a cow have at least three consecutive  $2 \times$  records or at least three consecutive  $3 \times$  records. The year or the herd in which the cow made her record was not copied from the original data file. Hence, the herd-to-herd and general year-to-year differences could not be measured separately. Requiring that the cow must have had three consecutive records on the same milking frequency must have excluded practically all cows from herds in which both  $2 \times$  and  $3 \times$  milking were being done at the same time. It seems likely that individual managemental or seasonal variations in environment which might have affected the regression of production on age would have been about the same within the  $2 \times$  and within the  $3 \times$  data. So large a sample of herds probably was fairly representative of HIR herds which are on test 4 consecutive yr. or more.

#### TREATMENT OF DATA

The change in production with increasing age was measured by the ratio between production at one age and production by the very same cows in their next lactation. The  $2 \times$  and  $3 \times$  data were studied separately but later were combined for deriving the age correction factors, since no certain difference was apparent except at ages under 36 mo. The two types of data provided 13,802 and 14,170 pairs of records.

The ratios were computed for every monthly age level in the data by assembling in one group all cows which had one lactation beginning at  $x$  months and another lactation beginning not more than 16 mo. later. Their total production ( $T_1$ ) in their lactations beginning at  $x$  months and their total production ( $T_2$ ) in their next lactations and the average interval ( $12 + m$ ) in months between the beginning of the first and the beginning of the next lactations were computed. Then  $T_2$  was adjusted to  $T$  for an interval of exactly 12 mo. by a linear interpolation thus:

$$(1) \quad T = T_1 + \frac{12}{12 + m} (T_2 - T_1).$$

Then  $R$ , the desired ratio of production at one age to what it was 12 mo. earlier was obtained by dividing (1) by  $T_1$  as is shown in equation (2). This was convenient for computing.

$$(2) \quad R = \frac{T}{T_1} = 1 + \frac{12}{12 + m} \left( \frac{T_2}{T_1} - 1 \right)$$

As a numerical example, the computations were as follows for the 284  $2 \times$  pairs in which the first records began when the cows were 24 mo. old:

$$\begin{aligned} R &= \frac{T}{T_1} = 1 + \frac{12}{12.5} \left( \frac{296,469}{262,779} - 1 \right) \\ &= 1 + 0.96 (1.13 - 1) \\ &= 1.12 \end{aligned}$$

This  $R$  shows the slope of the regression of production on age ( $x$ ) through the relation:  $R - 1 = \frac{\Delta y}{y} \div \frac{dy}{dx} \cdot \frac{\Delta x}{y}$  where  $y$  is the yield at the age when the first record of the pair began but  $\frac{dy}{dx}$  applies to the curve approximately 6 mo. later and  $\Delta x$  is 12 mo. As long as  $R$  exceeds 1.0,  $\frac{dy}{dx}$  is positive and production is still increasing with age. At the age of maximum production  $\frac{dy}{dx}$  is 0 and  $R$  is 1.0. When production starts to decline,  $R$  becomes less than 1.0. In these data the average value of  $m$  was a little less than 1 mo.

The numbers of record pairs at the various monthly age levels ranged from one to 331 in the  $2 \times$  data and from one to 353 in the  $3 \times$  data. In the combined  $2 \times$  and  $3 \times$  data the number of pairs varied from one to 665. The first records of the pairs were made at ages ranging from 16 to 182 mo. The pairs beginning under 24 mo. or later than 104 mo. were so few that the ratios at these ages could vary widely just by chance. This was minimized by using moving averages and by combining the ratios at extreme ages, as is explained later. In one-fifth of the pairs the first ages were under 36 mo. Half of them began at ages under 50 mo. A fifth of them began at ages as old as 72 mo., but only one-twentieth of the information was for pairs beginning at ages as high as 104 mo. At the youngest age and at the ages so old that the data were scarce, enough consecutive months were combined to have at least 20 cows as the basis for each  $R$  in the  $2 \times$  data. The same ages were used for grouping the  $3 \times$  data.



To reduce the effects of sampling fluctuations, a 5-mo. weighted moving average was fitted to both the  $2\times$  and  $3\times$  ratios. The relative weights for the 5 mo. were 1-3-4-3-1 in the  $2\times$  data, but the slightly different weighting, 1-2-3-2-1, was used later in the  $3\times$  and combined data, as it was found simpler to compute and the choice of weights had to be somewhat arbitrary anyhow. In all the weighting the ratio for each month was multiplied by the number of record pairs involved. This gave weight to the amount of evidence on which each  $R$  was based. These weighted average  $R$ 's are shown in figure 1.

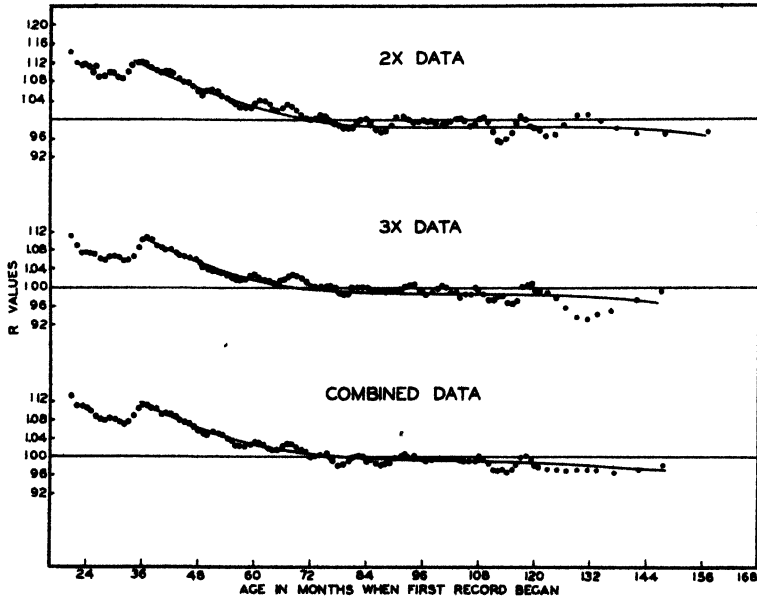


FIG. 1. Ratio of production at one age to production twelve months earlier.

The salient things about figure 1 are (a) the smoothness and simplicity of the curve for ages over 36 mo. and (b) the distinct sag between 24 and 36 mo. These things are so nearly alike in the  $2\times$  and the  $3\times$  data that they must be real and are not mere sampling accidents. That the up-and-down scatter of the points is slight, except at ages past 108 mo. where the data are few, indicates that only small sampling errors remain here.

That  $R$  changes in a slightly undulating way is partly a consequence of using moving averages. Adjacent months have six of their nine elements alike where the 1-2-3-2-1 weighting was used, points with 1 intervening month have four elements alike, those with two intervening have two elements alike, and even those with 3 intervening months still have one of their nine elements in common. But points with 4 or more intervening months are entirely independent, except as a second record in one pair was sometimes an initial record in some later pair.

What the sag before 36 mo. means concerning the shape of the age curve is

shown in figure 2 in the curve for "present" factors. Its plausible biological explanation will be discussed later.

The  $2 \times$  and the  $3 \times$  data in figure 1 follow a closely similar course, although the  $2 \times R$ 's are distinctly larger than  $3 \times R$ 's at ages under 36 mo. The  $2 \times R$ 's are a little larger than the  $3 \times R$ 's at ages from 36 to about 63 mo., but this seems too uncertain and too small to warrant constructing separate sets of correction factors. The difference means that under 36 mo. the  $2 \times$  curve should be a bit steeper and the  $3 \times$  curve correspondingly not quite as steep as the curve shown in figure 2 for "present" factors. A similar but extremely small difference occurs from 36 mo. to a little after 60 mo. The reason for the difference at ages under 36 mo. is apparent in figure 4.

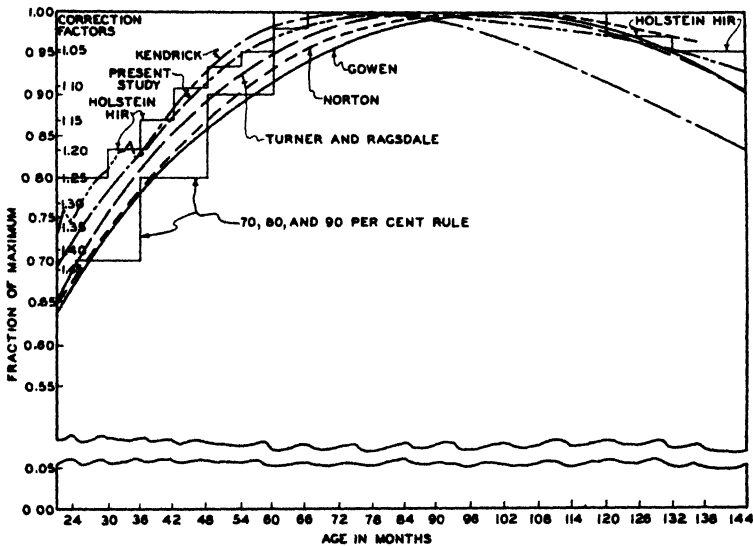


FIG. 2. The regression of production on age, according to several sets of correction factors.

The smooth curves in figure 1 for ages from 37 mo. onward were fitted by eye because of uncertainty about what type of equation to choose for fitting by precise mathematical methods and because unsatisfactory results were obtained, especially near the ends, when trying to fit polynomial curves.

The factors presented in table 2 and used in figures 2 and 3 were derived from the smooth curve fitted to the combined  $2 \times$  and  $3 \times$  data for ages of 37 mo. and over. The first step in deriving these factors was to find the point of maximum production,  $M$ , on the age-production curve. This would be about 6 mo. later than the age at which  $R$  becomes 1.0. Figure 1 makes it clear that  $M$  could not have been earlier than 78 nor later than 84 mo. This has been taken to be 82 mo., but  $R$  is so nearly 1.0 for quite a distance here that mistaking the exact value of  $M$  by as much as 4 mo. would have made only tiny differences in the age curve. The factor for converting to their mature equivalents the records begun at the age  $M$  minus 12 mo. is  $R$  at the age  $M$  minus 12 mo. The factor

for converting records begun at age  $M$  minus 24 mo. is found by multiplying  $R_{M-12}$  by  $R_{M-24}$ . The factor for  $M$  minus 36 mo. is given by the product,  $R_{M-12} \cdot R_{M-24} \cdot R_{M-36}$ . The factor for  $M$  plus 12 mo. is the reciprocal of  $R_M$ , that for  $M + 24$  is the reciprocal of  $R_M \cdot R_{M+12}$ , etc. This step-wise procedure was used to locate points at 12-mo. intervals on each side of  $M$ .

Points on the age curve at  $M - 3$ ,  $M - 6$  and  $M + 3$  were then located by considering the apparent general shape of the curve on both sides of  $M$  and interpolating. Then points at successive 12-mo. intervals on each side of  $M - 3$ ,  $M - 6$  and  $M + 3$  were found by using the  $R$  values read from the curve in figure 1, just as was done for locating the points at 12-mo. intervals from  $M$ . This gave at 3-mo. intervals the points on an age curve all the way from 37 mo. to 160 mo., although the scantiness of data at the advanced ages makes a considerable uncertainty about points for ages much past 10 yr. A smooth curve was fitted by hand to these points 3 mo. apart. Thus, was obtained the "present" curve shown in figure 2 for ages 37 mo. and over.

The points for 36 mo. and under were obtained by applying the actual moving average shown in the combined data in figure 1 to the point on the smoothed curve at the age exactly 12 mo. later. This seemed the only feasible way to treat the sharp discontinuity which is so prominent in figure 1 at or just before 36 mo.

#### RESULTS CONCERNING AGE

The factors thus derived are in table 2. Figure 2 shows these factors in comparison with some of the other previously published and widely used factors which have been derived from Holstein data.

As a rough and imperfect but easily understood test of how these various factors actually fit the HIR data, they were applied to the averages in table 1. The results are shown in figure 3. If (a) the factors were entirely correct and (b) the average intrinsic producing abilities really were the same for all the age-groups in table 1, the corrected curves in figure 3 should be horizontal except for minor sampling irregularities permitted by the averages being based on only moderate numbers of cows. But condition (b) is not expected to hold exactly. Individual culling probably will have made the cows in the older groups average somewhat higher in their production. This would make accurately corrected lines rise a little with increasing age. This rise might have been offset in part by a general increase in the average intrinsic productivity of the breed whereby, for example, the average productivity of all young cows born in 1935 would have been higher than the average of all those born in 1930. A few other, probably minor, circumstances might keep perfectly corrected lines from being absolutely horizontal.

#### RESULTS CONCERNING FREQUENCY OF MILKING

Figure 4 shows the  $2 \times 3 \times$  ratios by 3-mo. intervals. Only the first records from all the pairs were used. This assured that none of the records in figure 4 would have been terminal records of the cow, such as might possibly have been affected by any udder trouble or other ill health just preceding her death or

culling. For example, the 735 2× cows who freshened at ages 23, 24 or 25 mo. and had another 2× record beginning not more than 16 mo. later, averaged 9,263 lb. of milk in their records begun at an average age of 24 mo. The 561 3× cows who freshened at ages 23, 24 or 25 mo. and had another 3× record beginning not more than 16 mo. later, averaged 11,691 lb. of milk in their first lactation. The ratio of this 9,263 to 11,691 is the 0.792 shown.

TABLE 2

*Age correction factors from the present study (not smoothed for ages under 37 mo.)*

Age	Factor	Age	Factor	Age	Factor
(yr.-mo.)		(yr.-mo.)		(yr.-mo.)	
1-9	1.36	4-3	1.07	10-4 to 10-9	1.04
10	1.34	4	1.06		
11	1.34	5	1.06		
2-0	1.33	4-6 to 4-8	1.05	10-10 to 11-2	1.05
1	1.31				
2	1.28				
2-3	1.26	4-9 to 4-11	1.04	11-3 to 11-7	1.06
4	1.25				
5	1.24				
2-6	1.23	5-0 to 5-2	1.03	11-8 to 11-11	1.07
7	1.22				
8	1.20				
2-9	1.20	5-3 to 5-6	1.02	12-0 to 12-3	1.08
10	1.21				
11	1.21				
3-0	1.21	5-7 to 6-0	1.01	12-4 to 12-6	1.09
1	1.20				
2	1.18				
3-3	1.17	6-1 to 7-8	1.00	12-7 to 12-10	1.10
4	1.16				
5	1.15				
3-6	1.14	7-9 to 8-9	1.01	12-11 to 13-0	1.11
7	1.13				
8	1.12				
3-9	1.11	8-10 to 9-7	1.02	13-1 to 13-3	1.12
10	1.11				
11	1.10				
4-0	1.09	9-8 to 10-3	1.03	13-4	1.13
1	1.08				
2	1.08				

The averages in figure 4 are all for 3-mo. periods except the very first one and the last five. For these six points wider time intervals were pooled in order to secure larger numbers. For example, the very first ratio shown is that between the averages of 133 cows milked twice daily and 46 cows milked three times daily, freshening at ages 17 to 22 mo. Their freshening ages averaged barely under 21 mo. At the other extreme the ratio at the oldest age is that between 31 2× cows and 27 3× cows freshening at ages from 12 yr. and 8 mo. to 15 yr. and 2 mo. and averaging 13 yr. and 6 mo.

The amount of evidence bearing on each ratio is considered as proportional to the harmonic mean of the two numbers involved and is indicated roughly on

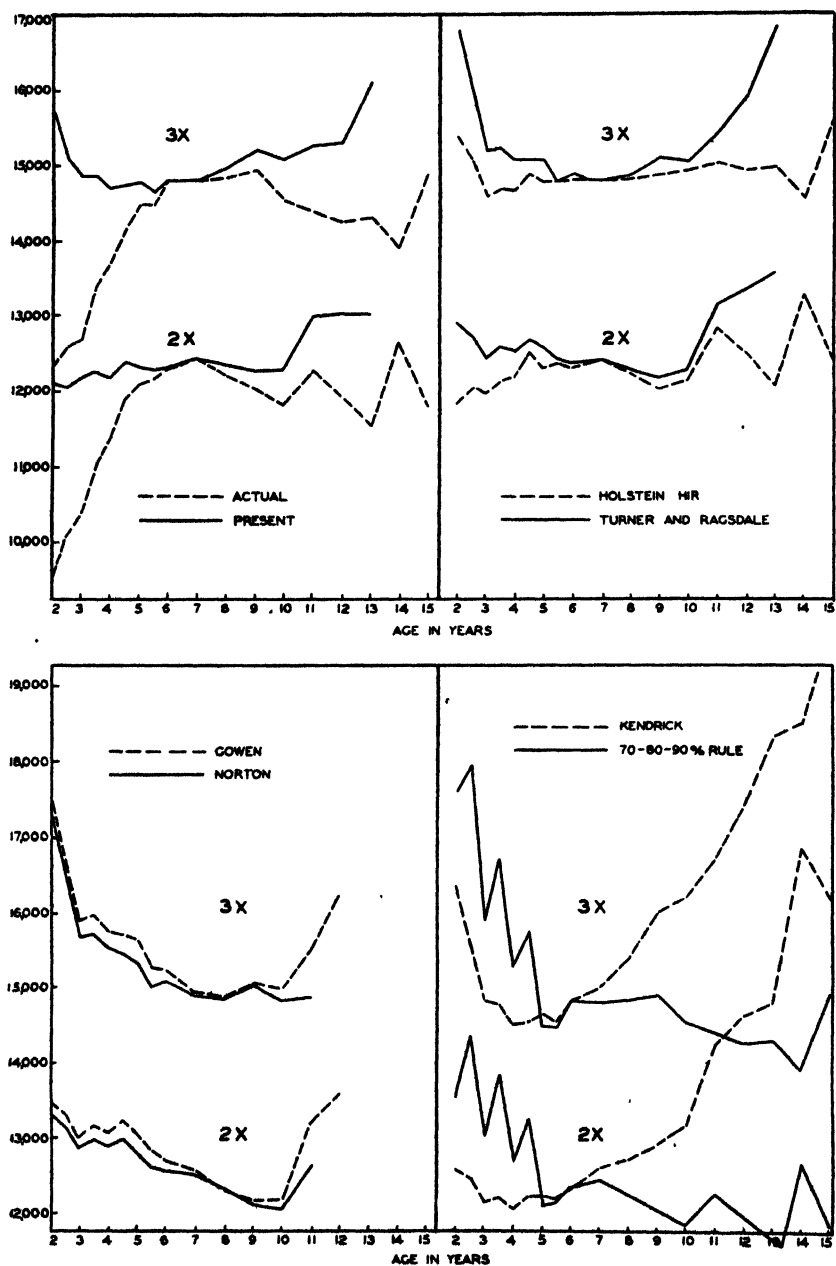


FIG. 3. The data of Table 1, actual and when corrected by the factors in Figure 2.

the graph. Only the last point is based on less evidence than the equivalent of 64 cows on each kind of milking. Two of the earlier points contain more evidence than if there were 900 cows on each milking frequency.

The horizontal line in figure 4 is at 0.833 which is the 5:6 rule so widely used for correcting  $3 \times$  to  $2 \times$  milking. This 0.833 seems to fit these data at 36 mo. and older ages well enough that practically nothing would be gained by changing it.

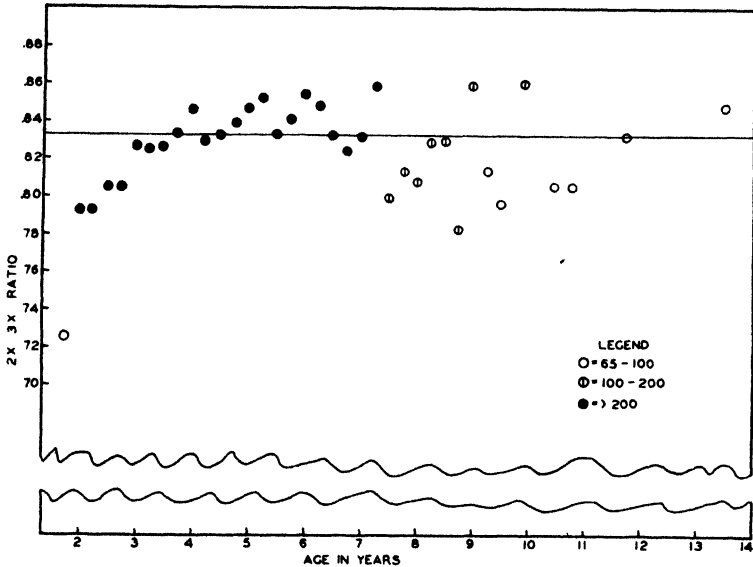


FIG. 4. Production by cows milked twice daily, compared with production by other cows the same age but milked three times daily.

At ages younger than 36 mo. the  $2 \times$  averages are distinctly less than five-sixths of the  $3 \times$  averages. The consistency of this and the amount of data make it certain that a different  $2 \times : 3 \times$  correction is needed at ages under 36 mo. Using the factor 0.80 in first lactations and 0.833 in all other lactations to correct  $3 \times$  records to a  $2 \times$  basis would fit these data rather well<sup>5</sup> and would be simple enough for widespread general use. It might be imagined that the difference found in the  $2 \times : 3 \times$  ratio at the young age is peculiar to 365-day records, many of which were in the present study, and that it does not occur in 305-day records. First lactations are known to be more persistent than later ones. But it seems more plausible that the extra milking generally would affect the first part of a heifer's record more than the latter part. This would follow if the extra milking affects yield primarily by relieving pressure in the udder. The udder capacity is relatively undeveloped at the beginning of the first lactation and would handicap production more in the first 305-days than it would in

<sup>5</sup> However, there is a strong hint that a perfectly accurate ratio would be progressively smaller at younger ages, especially in first records beginning at ages under 24 mo.

the 11th and 12th month. If that is an important part of the picture, 305-day lactations are likely to show an even more extreme difference in the  $2 \times : 3 \times$  ratio than was found here between early lactations and later lactations.

#### DISCUSSION

All the correction factors in figure 3 over-correct the  $3 \times$  records at ages under 36 mo. more strongly than they do the  $2 \times$  records. This bias appears to result from the extra milking having more effect on heifer lactations than on later ones. Therefore, in their first lactations, the  $3 \times$  cows need less age correction than the  $2 \times$  cows do. This situation can be met by having a single set of age correction factors suited to  $2 \times$  data and then using a smaller ratio for converting  $3 \times$  first lactations to a  $2 \times$  basis than is used for  $3 \times$  later lactations. If uncertain whether the lactation was a first or a second one, there would be small error in presuming it to be a first one if it began at an age less than 36 mo.

The "present" curve in figure 2 shows what the peak in the  $R$  curve (figure 1) at around 36 mo. really means. The most plausible explanation for this dip in the age curve is that cows which freshened young the first time begin to freshen for the second time in considerable numbers at around 35 mo. before they have completed their body growth or restored their nutritional reserves. Up to 34 mo. nearly all of the lactations are first calvings and the "present" curve in figure 2 shows the age change, unconfounded with any effects of prior milk production. But the second lactations begin to occur in appreciable numbers at ages of around 34 mo. By 37 mo. most of the data are for second lactations. This explanation is supported by the frequency of the combined  $2 \times$  and  $3 \times$  record pairs at the early ages, as shown in table 3.

TABLE 3  
*Distribution of record pairs by age at which the first record of the pair began*

Age	No. of pairs	Age	No. of pairs
(mo.)		(mo.)	
16	1	31	399
17	7	32	377
18	15	33	290
19	16	34	313
20	28	35	396
21	32	36	515
22	81	37	604
23	226	38	585
24	473	39	635
25	597	40	665
26	617	41	616
27	626	42	574
28	624	43	578
29	504	44	529
30	462	45	477

The first peak at ages of 25 to 28 mo., the valley at ages 31 to 35 mo. and the second peak at ages 37 to 41 mo. all testify to the cyclical distribution of calving ages among cows up to at least their third calving. This distribution of calving

ages also conforms to the report by Plum and Lush (7) and to some unpublished data we have seen recently from the Jersey HIR.

The evidence on the shape of the age curve up to about 34 mo. is practically all based on first lactations compared with second ones. The heifers are progressively nearer to their physical maturity when they do calve the first time. At about 34 mo. the comparisons between second and third lactations begin to occur and these all pertain to cows who freshened unusually young, both the first and the second time. Because they begin their second lactations still very immature, and in many cases somewhat depleted nutritionally, their second lactations are much smaller than their third ones, and thus they yield high  $R$  values at the ages when the early second lactations are beginning. The sharp rise in the  $R$  value (figure 1) at ages 34 to 37 mo. is caused by the early second calvings replacing the first calvings in the evidence for that period. Then, as the age at second calving gets much later than 37 or 38 mo., most of the cows will enter on their second lactations more nearly mature and having had more time to recuperate from their first lactations. Consequently the  $R$  values fall rapidly after about 37 mo. as they begin to be based on comparing third with second lactations which were made when the cows had recovered more completely from their first lactations. In the  $2 \times$  data there is a hint of a similar but much smaller irregularity in the  $R$  curve at about 50 to 52 mo., when comparisons between third and fourth calvings would begin to occur, but the  $3 \times$  data do not show this.

This explanation of the sharp break in the age curve at near 35 mo. fits extremely well the findings of Johansson and Hansson (3) (see especially the graph on their page 39), who studied the regression of butterfat yield on age within first, second, third and later lactations, separately. They found that cows calving a second time at an early age produced distinctly less than those calving for the first time at that same age.

It appears, therefore, that corrections for age will be more accurate if different age correction factors are used for first calvers and for second calvers during the ages when both are present in appreciable numbers. Probably this should be done, although the amount of work and confusion involved in changing any widely used factors is so much that the magnitude of the change needs to be verified first on other bodies of data. If a change had to be made now, the simplest way to do it seems about as follows: (a) The "present" curve in figure 2 would be given the very little bit of smoothing it needs for the youngest ages up to and including 34 mo. and would be lowered a bit at those ages to make it adapted to the  $2 \times$  data alone, rather than to an average of the  $2 \times$  and  $3 \times R$ 's as at present. (b) The factor for 35 mo. would be lowered enough to make it smooth with the existing curve for 36 mo. and later. (c) The resulting factors would be used with the qualifications (1) that if a first record began at age 35 mo. or later 0.04 would be subtracted from the factor listed for that age and (2) that if a second lactation began at ages 34 mo. or earlier, 0.05 would be added to the standard correction factor which would have been used if it were a first lactation. In effect this would make the standard corrections be for first



lactations at ages of 34 mo. and younger, but for second or later lactations beginning at 35 mo. or older. This would be fairly simple in operation, since the age range in which this amounts to using two sets of correction factors would be only from about 32 to about 40 mo. When it is not known whether the lactation was a first or a second one, the standard factor for that age would be used. This would be unfair to the few unknown second calvers at 34 mo. or less and it would be unduly generous to a few unknown first calvers at 35 mo. and older, but the net accuracy would be higher than using a single set of factors, regardless of lactation order, which is unfair to all early second calvers and unduly generous to all late first calvers. If the dividing age were set 1 mo. or even 2 mo. later than between 34 and 35 mo., fewer late first calvers would get an undeservedly high correction factor, but more second calvers would be unfairly penalized. This applies only to those for which it is not known whether the lactation was a first or a second one.

The AR factors (Gowen, Norton and Turner and Ragsdale) in figure 2 show the maximum to be at a later age and they show the production at the younger ages to be relatively lower than the HIR and DHIA factors indicate. The selective nature of AR testing is enough to explain this difference. Many of the men doing AR testing would follow a general policy of testing young cows at the first convenient and promising lactation but would think it worth while to test older cows only when those seemed likely to make an outstanding record, or at least a record much higher than any they already had. That is, in AR testing there is not only selecting of cows to be tested but also some selecting of their most promising lactations. This latter kind of selection naturally would be more extreme among older cows, since they would have started more lactations.

As far as concerns any bias from selection, the Kendrick and "present" and Holstein HIR curves show the true age curve more accurately than the AR curves do, although the former two may show the maximum at slightly too young an age and be a bit too flat before that and may decline a bit too rapidly afterward, because of the bias inherent in method B. Four of the seven sets of factors appear to over-correct at least slightly at ages past 10 yr., as judged by the evidence in figure 3. The Norton and the Holstein HIR factors have little, if any, of this fault and, of course, the 70-80-90 per cent rule makes no corrections there. This over correction at older ages is most extreme with the Kendrick factors. The practical importance of age correction factors being wrong for these ages is small because so few records are made by cows this old. If the lifetime averages of those cows are used, as they should be, errors in the correction factors at ages as old as 10 yr. will lead to only tiny errors in estimating the productivity of the cows, except in those rare cases where a cow is already old when testing begins in the herd. Many of the Kendrick data came from DHIA herds which were not on HIR test, but it seems unlikely that average management or culling practices could have been so different between DHIA and HIR herds that they would be the sole causes of the Kendrick factors showing production to decline so much at the older ages. That would require that old cows be given much worse management than young or middle-aged cows in

DHIA herds but not in HIR herds, or that culling of genuinely poor producers among the cows past middle age is far more severe and effective in DHIA herds than in HIR herds. We see no reason to suppose the former to have been true to any considerable extent, while for the latter to have been the major cause of the discrepancy seems impossible in view of the limitations which reproductive rates set on the culling possibilities among cows. One trying to cull enough to make so large a change in genuine productivity would find himself practically out of cows in 2 or 3 yr. at most! It is not apparent that any difference between 305-day and 365-day records could have been responsible for this difference at the older ages; indeed, it seems that any tendency for cows to become less persistent as they grow old would have operated in the other direction. Possibly the Kendrick data include more pairs in which the second record was really a terminal one, abnormally small because of udder trouble or ill health, but we see no reason why such pairs should be much more frequent in DHIA than in HIR data at older ages but not more frequent at younger ages.

As compared to the present and the Holstein HIR factors, the Kendrick ones appear slightly too low at ages of about 45 to 66 mo., as can be seen in figure 2. This is confirmed in figure 3 for the  $3 \times$  data and, at least for the junior fours, in the  $2 \times$  data. The difference is small but, because it pertains to ages at which many records are made, it deserves inquiry whenever a revision of the present Kendrick factors is considered.

It is easy to exaggerate the importance of differences in age correction factors and to waste time on further refinements of those, while neglecting things which cause much larger errors in selections and cullings. As an approximate method of measuring the actual importance of age correction factors, the variance component due to differences between age groups in the uncorrected  $2 \times$  data of table 1 was estimated and then compared with the amount that remained in these data after they had been age-corrected as shown in figure 3. The age component in the uncorrected data of table 1 was about 11 per cent of the individual variance. On account of the absence of young cows with only one or two records from these data and the under-representation of cows with records at extremely old ages, we suppose that 14 to 16 per cent would be a better estimate for the general importance of age in causing variations in utterly unselected records. Also some real age differences still remain in table 1 within such broadly based groups as junior twos and threes and senior twos, so that the 11 per cent found is not quite all of it. In the unselected Johansson and Hansson data (their table 5) the component between groups was also only 11 per cent of the total. But then their data were grouped only by lactation order, so that a larger share of the age variance must have remained within their groups than within the groups in table 1 here.

The simple 70-80-90 per cent rule takes out 52 per cent of the age variance which was in table 1. That such crude factors could remove so much of the age effect indicates already that even a few refinements would go far toward completing the job. The extremely step-wise nature of the 70-80-90 per cent corrections causes the sharp zigzags at the younger ages in figure 3. These cor-

rections are clearly too generous to the 2-yr.-olds and 3-yr.-olds, especially to the senior ones among them.

The Kendrick factors take out 91 per cent of the age variance. Half of the 9 per cent which is left comes from the factors being too high at ages of 11 and over, while two-thirds of it comes from their being too high at ages of 9 and over. Errors at those older ages will cause little practical damage, since such cows will already have other records except in the few cases where they are in herds which are just beginning to test. About 2 out of the 9 per cent of the original age variance left by the Kendrick corrections comes from those factors being too small in the age zone from junior threes to senior fives. This 2 per cent is certainly too small to justify the confusion and clerical work of changing factors until it has been verified on other data, yet it does have a little practical importance because so many culling and selection decisions must be based on records made in that age zone.

The Holstein HIR factors retain only about 3 per cent of the original age variance and the "present" ones retain only 1 per cent, but such figures show these factors in rather too good a light, since they are being tested back against the very data from which they were wholly (the "present" factors) or in large part (the Holstein HIR factors) derived. Nearly half of the Holstein HIR discrepancy comes from the coarse grouping for junior twos and about a fifth of it comes from the factor being too large for the senior fours.

Except for the  $3 \times 2$ -yr.-olds and to a much lesser extent the  $3 \times 3$ -yr.-olds, the "present" factors give in figure 3 a slight upward slope such as would be expected, either from the bias in the method or from selective elimination of the cows having made the older groups in table 1 genuinely more productive than the younger ones. The upward trend seems a bit more extreme than the latter cause alone seems likely to have produced. Part of the upward slope in the very youngest ages in the  $2 \times$  data, as well as the downward slope in the youngest ages in the  $3 \times$  data, is due to our having combined the two kinds of data to derive an average *R* curve before we learned that the  $2 \times : 3 \times$  ratio was smaller at the younger ages, especially at 36 mo. and less. The upward trend expected because of having used paired records is shown by the Kendrick factors only from junior fours onward. Why it does not appear earlier is not clear and it can hardly be the major cause of the Kendrick curves going so extremely high at ages after six.

The step-wise nature of the Holstein HIR factors introduces some moderately important discrepancies at ages less than 48 mo. Whenever these factors are revised the steps should be shortened, at least to 3-mo. intervals. With modern computing machinery the clerical workers in the breed office would probably find little more work in using a separate factor for each month than in using a separate factor for each 3-mo. interval. The gain in accuracy from using the monthly factors would be trifling at ages past 48 mo.

How little is actually gained by extreme refinement of correction factors can be illustrated with fat production, which is only a little lower than milk in repeatability and in coefficient of variability. With an intra-herd standard

deviation of about 80 lb. of fat and a repeatability of about 0.4 in age-corrected 305-day lactation records, the standard deviation of a cow's individual records around her true productive value is  $80\sqrt{0.6}$ . One quarter of this is about 16 lb. The standard error of estimating her real ability from one record is  $80\sqrt{0.4\cdot\sqrt{0.6}}$ , one fourth of which is about 10 lb. Little information is lost by grouping data, provided the grouping interval is as small as one-quarter of a standard deviation. Hence almost no accuracy in estimating the future production of a cow is gained by considering her individual lactation fat record in any more detail than rounding it to the nearest 10 lb. To make that much difference on an actual record of 500 lb. of fat, the age-correction factor would have to be in error by about 0.02.

Small biases from age-correction factors can become important, however, when comparing the averages of groups differing widely in average age, as may often be the case in proving a sire by comparing the production of his daughters with that of their dams. For example, if nine cows were in each age group, the statistician's rough rule would give about 5 lb. (instead of 16) as the largest interval in which such averages ought to be grouped, while if there were 100 cows in each group that interval would sink to about 1.6 lb. or a little less. For such purposes errors even as small as 0.01 in the correction factors occasionally attain some practical importance. Of course there is no argument for leaving even small errors in the correction factors unless the cost of removing them (including the confusion caused by any change in factors already widely used) costs more than the gain in accuracy is worth.

Age corrections by regression, rather than by multiplicative factors have been proposed sometimes (10). These really attempt in a single operation to correct *both* for age and for imperfect repeatability, rather than for age alone as the multiplicative factors do. Gowen (2, table 23) showed long ago that the standard deviation of actual records varies from age to age nearly as the mean does. Therefore, a multiplicative correction comes nearer than a regression equation to making the corrected records equally variable at all ages. Without that equality, records corrected to maturity cannot fairly be pooled with each other or with actual records made at maturity. Allowance for imperfect repeatability does need to be made in most practical situations but usually it is simpler and more accurate to correct for age in one operation and then for imperfect repeatability in another.

The present analysis sheds no light on the argument concerning correcting for weight instead of correcting for age (1), since weights were not available. In any event, weight and age are confounded in most data so that correcting on either basis does part (although not all) of what would be done by correcting on the other.

Only the milk records were studied here, whereas some of the other studies were of fat. But milk quantity changes so much more than test does with age and with times milked that separate sets of correction factors for milk and for total fat do not seem worth while. During the latter part of the lactation the test increases slightly. This might possibly have effect enough to make some de-

tectable difference between 365-day milk factors and 305-day fat factors, especially in first lactations. Test decreases very slightly with age but this does not seem enough to have accounted for any detectable part of the difference discussed.

#### SUMMARY

1. Multiplicative age-correction factors for Holsteins were derived from 43,573 Herd Improvement Registry lactations of 5,374 cows milked twice daily and 5,627 cows milked thrice daily. These factors were based on comparisons of production by the same cows in consecutive lactations. The factors are shown in table 2.

2. The biases which culling of cows may cause in age correction factors are discussed. Several sets of factors for Holsteins are compared in figure 2. Figure 3 shows the actual HIR averages before and after correction of these factors.

3. The present findings agree well with the widely used Kendrick factors. The differences indicate that the following four points need attention whenever a revision of the Kendrick factors is made:

- (a) The Kendrick factors seem a bit too large at ages under 35 mo. Part of this might come merely from the higher persistency of heifers, since the present data included many lactations which went as long as 365 days. Part of it may have come from neglecting the difference between late first calvers and early second calvers in the smoothing when the Kendrick factors were prepared.
- (b) Some difference needs to be made between late first and early second calvers at those ages when records by both are present; *i.e.*, at around 33 to 42 mo. This might be done rather simply by having a sharp discontinuity in the standard age-correction factors between 34 and 35 mo. and adding about 0.05 to the standard factor if the calving is a second one which occurred earlier than 35 mo., but subtracting about 0.04 from the standard factor if it is a first calving which occurred at ages later than 34 mo.
- (c) The Kendrick factors appear a bit too low at ages around 45 to 66 mo. The apparent difference is small but does have a little importance, because so many of the records on which cows are culled or are chosen to be the dams of herd sires are made at these ages.
- (d) The Kendrick factors seem decidedly too large at the older ages.

4. Because all of the commonly used age corrections remove most of the variance caused by age, their accuracy can be increased only a little by further changes. Since any changes involve much confusion and clerical labor, those which the present study indicates to be desirable should first be verified on other data, especially with respect to these points:

- (a) Is any material portion of them the peculiar result of the present data having included many lactations as long as 365 days?
- (b) Is the slope of the age curve really different enough in HIR and in DHIA data to need attention?

- (c) Is the explanation proposed here for the peculiar shape of the  $R$  curve at around 36 mo. really valid and is there any simpler and equally accurate device for adapting age corrections to it?

5. Multiplying  $3 \times$  records by 0.80 when they are first lactations and by 0.833 when they are later lactations is suggested for correcting them to the basis of twice-a-day milking. The data hint that the difference between  $2 \times$  and  $3 \times$  milking is even larger than 4:5 at extremely young ages.

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PROGRAM  
FORTY-FIFTH ANNUAL MEETING  
OF THE  
AMERICAN DAIRY SCIENCE ASSOCIATION  
CORNELL UNIVERSITY  
ITHACA, NEW YORK  
JUNE 20-22, 1950

GENERAL PROGRAM COMMITTEE

W. E. PETERSEN, Minnesota, <i>Chairman</i>	G. M. CAIRNS, Maryland
C. W. REAVES, Florida	J. M. SHERMAN, New York
D. V. JOSEPHSON, Pennsylvania	K. L. TURK, New York

GENERAL PROGRAM

*Monday, June 19, 1950*

12:00 Noon\*      **Open for Registration, Memorial Room, Willard Straight Hall**

7:00-12:00 p.m.    **Registration and Informal Gathering, Memorial Room, Willard Straight Hall**

*Tuesday, June 20, 1950*

8:00 a.m.            **REGISTRATION**

10:00-12:00 a.m.   **OPENING SESSIONS, Bailey Hall**  
                         K. L. TURK, *Department of Animal Husbandry, Cornell University*, presiding

**Address of Welcome**  
                         DR. W. I. MYERS, *Dean, College of Agriculture, Cornell University*

**Presidential Address**  
                         DR. G. M. TROUT, *Dairy Division, Michigan State College*

**Guest Speaker**  
                         DR. E. E. DAY, *Former President and Chancellor, Cornell University*

1:30- 4:30 p.m.    **SECTIONAL MEETINGS**  
                         **Production Section A**  
                         Genetics  
                         *Room 100, Caldwell Hall*

\* All events scheduled are Eastern Daylight Saving Time.

**Production Section B**

Calf Nutrition

*Room 25, Warren Hall*

**Manufacturing Section**

Chemistry

*Room 233, Plant Science Building*

**Extension Section**

Opening Business Session, Teaching Methods and Exhibits, *Room 125, Warren Hall*

Extension Exhibits, *Room 101, Warren Hall*

8:00-12:00 p.m. RECEPTION AND DANCE, *Statler Hall*

*Wednesday, June 21, 1950*

9:00-12:00 a.m. SECTIONAL MEETINGS

**Production Section A**

Endocrines and Mammary Secretions

*Room 100, Caldwell Hall*

**Production Section B**

Nutrition and Physiology

*Room 25, Warren Hall*

**Manufacturing Section**

Cheese, Whey

*Room 233, Plant Science Building*

**Extension Section**

Dairy Herd Improvement Associations and Dairy Cattle Breeding

*Room 125, Warren Hall*

1:30- 4:00 p.m. SECTIONAL MEETINGS

**Production and Extension Sections (Joint Session)**

Symposium: Grassland Utilization and Its Relation to Dairying

*Bailey Hall*

**Manufacturing Section**

Microbiology, Sanitation

*Room 233, Plant Science Building*

4:00- 5:00 p.m. COMMITTEE AND BUSINESS MEETINGS

**Production Section, Room 25, Warren Hall**

**Manufacturing Section, Room 233, Plant Science Building**

8:00-10:00 p.m. ENTERTAINMENT, *Bailey Hall*



*Thursday, June 22, 1950*

9:00–11:00 a.m. SECTIONAL MEETINGS AND BUSINESS MEETINGS

**Production Section A**

Feeding

*Room 100, Caldwell Hall*

**Production Section B**

Reproduction

*Room 25, Warren Hall*

**Manufacturing Section**

Milk, Ice Cream, Concentrated and Dry Milks

*Room 233, Plant Science Building*

**Extension Section**

4-H Club Work, Committee Reports, Business Meeting

*Room 125, Warren Hall*

11:00–12:00 a.m. **Production Section Business Meeting, Room 25, Warren Hall**

1:30–3:00 p.m. SECTIONAL MEETINGS

**Production Section A**

Management

*Room 100, Caldwell Hall*

**Production Section B**

Semen Techniques

*Room 25, Warren Hall*

**Manufacturing Section**

High Temperature Processing

*Room 233, Plant Science Building*

**Extension Section**

Dairy Cattle Health

*Room 125, Warren Hall*

3:00–5:00 p.m. **BUSINESS MEETING OF THE ASSOCIATION**

*Room 25, Warren Hall*

7:00 p.m. **ANNUAL BANQUET, INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS, Statler Hall**

**J. M. SHERMAN, Department of Dairy Industry, Cornell University, Toastmaster**

**PROGRAM OF ENTERTAINMENT**

(Principally for the Ladies)

*Tuesday, June 20, 1950*

3:00 p.m. **TEA, Martha Van Rensselaer Hall**

8:00–12:00 p.m. **RECEPTION AND DANCE, Statler Hall**  
(Open to all registered.)

*Wednesday, June 21, 1950*

- 1:00 p.m. COMPLIMENTARY LUNCHEON AND BRIDGE, *Statler Hall*  
 8:00 p.m. CONCERT. Endicott Johnson Workers' Chorus, *Bailey Hall*  
 Courtesy of Endicott Johnson Corp., Johnson City, N. Y.  
 (Open to all registered.)

*Thursday, June 22, 1950*

- 10:00 a.m. TOUR of Taughannock Falls and Robert E. Treman State  
 Parks. Complimentary picnic luncheon for ladies and  
 children  
 7:00 p.m. ANNUAL BANQUET, INSTALLATION OF OFFICERS AND PRESENTA-  
 TION OF AWARDS, *Statler Hall*. (Open to all registered.)

PROGRAM OF MANUFACTURING SECTION

*Tuesday, June 20*

Afternoon Session. *Room 233, Plant Science Building*

- 1:30- 4:30 CHEMISTRY. D. V. JOSEPHSON, *Chairman*  
 M1 Ion Exchange as a Means of Varying the Salt Constitu-  
 ents of Milk. H. S. HALLER AND A. G. MORIN, *Bu-  
 reau of Dairy Industry, U.S.D.A.*  
 M2 Isolation of the Non-casein Proteins of Milk. A. R.  
 KEMP, B. C. JOHNSON AND A. M. SWANSON, *University  
 of Wisconsin*  
 M3 A Detailed Study of the Non-protein Nitrogen Frac-  
 tions in Milk. K. M. SHAHANI AND H. H. SOMMER,  
*University of Wisconsin*  
 M4 A Colorimetric Determination of Lipase. (A Prelimi-  
 nary Report.) GEORGE R. GREENBANK AND PHILIP A.  
 WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*  
 M5 Nephelometric Determination of Fat in Non-fat Dry  
 Milk. BURDET HEINEMANN, E. J. BALDI AND O. B.  
 PARKER, *Producers Creamery Co., Springfield, Mo.*  
 M6 The Action of Mineral-ion Exchange Resins on Certain  
 Milk Constituents. CHARLES W. GEHRKE, *University  
 of Missouri*, and EMORY F. ALMY, *Ohio State Univer-  
 sity*  
 M7 Preliminary Observations on the Electrophoresis  
 Study of the Proteins in Skimmilk. W. L. SLATTER  
 AND Q. VANWINKLE, *Ohio State University*  
 M8 State of Solution of the Naturally Occurring Salts in  
 Milk. INDRAPAL S. VERMA AND H. H. SOMMER, *Uni-  
 versity of Wisconsin*

- M9 Studies on Oxidized Milk Fat. MARK KEENEY AND F. J. DOAN, *Pennsylvania State College*
- M10 The Enzymatic Hydrolysis of Lactose in Dairy Products and Its Determination. FRANK E. POTTER, *Agricultural Research Administration, U.S.D.A.*
- M11 Studies on the Water-insoluble Acids of Butter. F. J. BABEL, *Purdue University*
- M12 The Lactometer as an Instrument for Determining Added Water in Milk. OLE YSTGAARD AND E. W. BIRD, *Iowa State College*

*Wednesday, June 21*

Morning Session. *Room 233, Plant Science Building*

9:00-12:00

- CHEESE, WHEY. E. L. JACK, *Chairman*
- M13 Rapid Salting of Brick Cheese. H. J. BUYENS AND W. V. PRICE, *University of Wisconsin*
- M14 Pasteurization of Milk for Italian Cheese Curd. J. C. MARQUARDT, *New York State Department of Agriculture and Markets*
- M15 The Influence of Ultrasonic Sound Waves on Cheese Ripening. W. C. WINDER, A. M. SWANSON AND W. V. PRICE, *University of Wisconsin*
- M16 Observations on an Exudate from Cheddar Cheese. V. L. ZEHREN AND A. M. SWANSON, *University of Wisconsin*
- M17 The Forced-drying of Cheddar Cheese Prior to Paraffining. D. M. IRVINE AND W. V. PRICE, *University of Wisconsin*
- M18 The Free Amino Acids of Foreign Type Cheese. F. V. KOSIKOWSKY AND A. C. DAHLBERG, *Cornell University*
- M19 The Methyl Ketones of Blue Cheese. STUART PATTON, *Pennsylvania State College*
- M20 Tyramine Production in Cheese and in Various Bacterial Cultures. JOHN A. HUPFER, JR., GEORGE P. SANDERS AND RALPH P. TITSLER, *Bureau of Dairy Industry, U.S.D.A.*
- M21 The Effect of Penicillin and Streptomycin on Swiss Cheese Starters. R. E. HARGBOVE, H. E. WALTER, J. P. MALKAMES AND K. T. MASKELL, *Bureau of Dairy Industry, U.S.D.A.*
- M22 Observations on a Gelatinous Curd Type of Spoilage of Cottage Cheese. R. B. PARKER, V. N. SMITH AND P. R. ELLIKER, *Oregon State College*

- M23 Low Cost and Small Scale Methods for Concentrating Whey for Feed. A. H. STEVENS, *Bureau of Dairy Industry, U.S.D.A.*

Wednesday, June 21

Afternoon Session. Room 233, Plant Science Building

- 1:30- 4:00 **MICROBIOLOGY, SANITATION.** J. H. HETRICK, *Chairman*
- M24 The Utilization of Whey in the Microbiological Synthesis of Riboflavin. ABRAHAM LEVITON AND EARLE O. WHITTIER, *Bureau of Dairy Industry, U.S.D.A.*
- M25 Strains of *Streptococcus faecalis* Present in a Starter Used in the Manufacture of Cheddar Cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, *Cornell University*
- M26 Bacterial Studies of the High-temperature Short-time Pasteurization of Ice Cream Mix. F. W. BARBER AND H. P. HODES, *National Dairy Research Laboratories, Inc.*
- M27 The Influence of pH on Proliferation of the Lactic Streptococcus Bacteriophage. W. W. OVERCAST, F. E. NELSON AND C. E. PARMELEE, *Iowa State College*
- M28 Changes in Bacteriophage and Sensitive Organism Populations in a Commercial Mixed Culture. C. E. PARMELEE, F. E. NELSON AND W. W. OVERCAST, *Iowa State College*
- M29 A Study on the Psychrophilic Bacteria in Market Milk. F. A. ROGICK AND L. H. BURGWALD, *Ohio State University*
- M30 Effects of Storage on Penicillin in Dairy Products. W. A. KRIENKE AND E. L. FOUTS, *University of Florida*
- M31 Organic Chelating Agents as an Aid to Dairy Detergency. G. A. CLAYBAUGH AND J. M. JENSEN, *Michigan State College*
- M32 A Comparison of Phosphatase Tests Using Different Buffers, Precipitants and Periods of Incubation. GEORGE P. SANDERS AND JOHN A. HUPFER, JR., *Bureau of Dairy Industry, U.S.D.A.*
- M33 Phosphatase Measurements on High Temperature Vacuum Pasteurized Churning Cream and Ice Cream Mix. G. H. WILSTER AND JUNIUS COVINGTON, *Oregon State College*
- M34 Factors Affecting Production of Proteolytic and Coagulating Enzyme by *Streptococcus liquefaciens*. A. T. DUDANI AND F. E. NELSON, *Iowa State College*

4:00- 5:00

**BUSINESS MEETING***Thursday, June 22*Morning Session. *Room 233, Plant Science Building*

9:00-12:00

**MILK, ICE CREAM, CONCENTRATED AND DRY MILKS.** E. L. JACK, *Chairman*

- M35** Taking Representative Milk Samples from Weigh Tanks. J. C. MARQUARDT, *New York State Department of Agriculture and Markets*
- M36** Determination of Quaternary Ammonium Compounds in Milk and in Detergent Sanitizer and Buffered Quaternary Solutions. D. D. MILLER AND P. R. ELLIKER, *Oregon State College*
- M37** Ion Exchange as a Means of Improving the Keeping Quality of Frozen Homogenized Milk. H. S. HALLER AND R. W. BELL, *Bureau of Dairy Industry, U.S.D.A.*
- M38** Observations on the Effect of Additions of Heat Thickened Protein to Fluid Milk on the Creaming Phenomenon. A. C. SMITH AND F. J. DOAN, *The Pennsylvania State College*
- M39** The Effectiveness of Some Antifoaming Agents in the Condensing of Skimmed Milk and Whey. J. ROBERT BRUNNER, *Michigan State College*
- M40** The Antioxidant Properties of Nordihydroguaiaretic Acid in Cream Pasteurized at Various Temperatures. B. T. KARNANI, DIONISIOS A. THEOKAS AND VLADIMIR N. KRUKOVSKY, *Cornell University*
- M41** Stability of Evaporated Milk as Influenced by Various Conditions of Homogenization. R. B. MAXOY AND H. H. SOMMER, *University of Wisconsin*
- M42** Separation of Fat and Protein in Sterilized Milks During Storage. B. H. WEBB, E. F. DEYSHER, C. F. HUFNAGEL AND F. E. POTTER, *Bureau of Dairy Industry, U.S.D.A.*
- M43** The Use of Concentrated Essence for Improving the Flavor of Strawberry and Peach Ice Cream. C. C. FLORA, L. L. DAVIS AND C. W. HOLDAWAY, *Virginia Agricultural Experiment Station*
- M44** Some Relationships of the Oxidation-reduction Systems of Milk to the Keeping Quality of the Dry Product. H. A. HARLAND, S. T. COULTER AND R. JENNESS, *University of Minnesota*
- M45** A Disc Method of Filtration for Roller Process Non-fat Dry Milk Solids. D. R. STROBEL AND C. J. BABCOCK, *Research Division, Dairy Branch, U.S.D.A.*

- M46 Comparison of the Yield of Non-fat Dry Milk Solids when Using Three Types of Spray Drying Equipment. BEN M. ZAKARIASEN AND GUNNER E. NELSON, *Land O'Lakes Creameries, Inc.*

*Thursday, June 22*

Afternoon Session. *Room 233, Plant Science Building*

- 1:30- 3:00 **HIGH TEMPERATURE PROCESSING.** D. V. JOSEPHSON, *Chairman*
- M47 Effect of Heating on the Diffusion of Calcium, Magnesium, Phosphorus and Citric Acid. T. D. HARMAN AND W. L. SLATTER, *Ohio State University*
- M48 The Operation of a Spray Drier at High Temperature and under Pressure. V. H. TOWNLEY AND S. T. COULTER, *University of Minnesota*
- M49 The Changes Produced in the Ultrafilterable Calcium, Phosphorus and Nitrogen Components of Skim Milk during Processing in a Mallory Heat Exchanger. E. A. BERNARDONI AND S. L. TUCKEY, *University of Illinois*
- M50 Effect of High-temperature Short-time Heat Treatments of Milk on the Denaturation of Albumin and Globulin. J. H. HETRICK, *Dean Milk Co.*, and P. H. TRACY, *University of Illinois*
- M51 Lactose Degradation in Heated Milk. STUART PATTON, *Pennsylvania State College*
- 3:00- 5:00 **BUSINESS MEETING OF THE ASSOCIATION**

**PROGRAM OF PRODUCTION SECTION**

*Tuesday, June 20*

Afternoon Session

- 1:30- 4:30 Section A. **GENETICS.** G. M. CAIRNS, *Chairman*  
*Room 100, Caldwell Hall*
- P1 A Study of the Inheritance of Persistency in Milk Production. M. H. ALEXANDER, *University of Illinois*
- P2 Some of the Effects of Calving Interval on Milk and Butterfat Production of Ayrshire Cattle. W. J. TYLER AND GEORGE HYATT, JR., *West Virginia University*
- P3 A Study of the Type Ratings of Daughters of Sires and Dams that have been Classified for Type. W. J. TYLER, *West Virginia University*
- P4 Preliminary Report on the Production Records of Crossbred Dairy Cattle. J. P. LAMASTER, G. W.

BRANDT AND C. C. BRANNON, *South Carolina Agricultural Experiment Station*, and M. H. FOHRMAN, *Bureau of Dairy Industry, U.S.D.A.*

- P5 Inheritance of Butterfat Test in the Beltsville Holstein Herd. JOSEPH B. PARKER AND CHARLES A. MATTHEWS, *Bureau of Dairy Industry, U.S.D.A.*
- P6 A Selection Index for Fat Production Utilizing the Fat Yields of the Cow and Her Relatives. J. E. LEGATES AND JAY L. LUSH, *Iowa State College*
- P7 An Index for the Effects of Certain Environmental Influences on Dairy Cattle Production. N. D. BAYLEY AND E. E. HEISER, *University of Wisconsin*
- P8 Heritability of Fertility in Dairy Cattle. R. S. DUNBAR, JR. AND C. R. HENDERSON, *Cornell University*
- P9 Predicting the Breeding Efficiency of Dairy Cows. DURWARD OLDS AND D. M. SEATH, *Kentucky Agricultural Experiment Station*
- P10 The Frequency Distribution of Cellular Antigens in Five Breeds of Dairy Cattle. L. C. FERGUSON, E. J. LAZEAR AND FORDYCE ELY, *Ohio State University*
- P11 Length of Gestation in the Five Major Breeds of Dairy Cattle. M. H. ALEXANDER, *University of Illinois*

1:30- 4:30

Section B. **CALF NUTRITION.** L. O. GILMORE, *Chairman*

*Room 25, Warren Hall*

- P12 The Preparation of a Riboflavin-deficient Milk for Experimental Calf Feeding. E. G. MOODY, S. M. HAUGE AND N. S. LUNDQUIST, *Purdue University*
- P13 The Influence of Rumen Inoculations on the Digestibility of Dry Matter, Cellulose and Protein in Young Dairy Calves. H. R. CONRAD, J. W. HIBBS, W. D. POUNDEN AND T. S. SUTTON, *Ohio Agricultural Experiment Station*
- P14 Variations in Digestibility of Certain Characteristic Rumen Microorganisms and Some Effects of their Absence on Calves. W. D. POUNDEN AND J. W. HIBBS, *Ohio Agricultural Experiment Station*
- P15 Some Chemical and Physical Characteristics of the Contents of the Alimentary Tract of Calves at Time of Birth. D. B. PARRISH AND F. C. FOUNTAINE, *Kansas Agricultural Experiment Station*
- P16 Postpartum Development of Bovine Stomach Compartments and Observations on Some Characteristics of their Contents. SIDNEY P. MARSHALL, P. T. DIX ARNOLD AND R. B. BECKER, *University of Florida*

- P17 The Growth of Dairy Calves on Purified Diets. G. P. LOFGREEN, *University of California*
- P18 The Nutritive Value of Starch and the Effect of Lactose on the Nutritive Values of Starch and Corn Syrup in Synthetic Milks for Young Calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN AND H. D. WEBSTER, *Michigan Agricultural Experiment Station*
- P19 Is A. P. F. of Value in a Calf Starter for Calves Weaned from Milk at an Early Age? L. L. RUSOFF AND M. O. HAQ, *Louisiana State University*
- P20 A. P. F. Supplements in Milk Replacements for Dairy Calves. J. B. WILLIAMS AND C. B. KNOTT, *Pennsylvania Agricultural Experiment Station*
- P21 Serum Protein Fractions of Calf Blood as Influenced by Colostrum and Skim Milk. R. F. ELLIOTT AND CECIL CONLEY, *Kentucky Agricultural Experiment Station*
- P22 The Effect of Kind of Carrier and Method of Dispersion on the Absorption of Carotene by Young Dairy Calves. J. W. CROWLEY AND N. N. ALLEN, *University of Wisconsin*
- P23 The Riboflavin Requirement of the Very Young Calf. GERMAIN J. BRISSON AND T. S. SUTTON, *Ohio State University*
- P24 Synthesis of Certain B-vitamins in the Digestive Tract of Dairy Calves. E. M. KESLER AND C. B. KNOTT, *Pennsylvania State College*

Wednesday, June 21

Morning Session

9:00-12:00

Section A **ENDOCRINES AND MAMMARY SECRETIONS.** G. M. CAIRNS, *Chairman*

*Room 100, Caldwell Hall*

- P25 The Effects of Estrogen and Progesterone on the Arterial System of the Uterus of the Cow. WILLIAM HANSEL, *Cornell University*
- P26 The Fertility of Heifers Following Administration of Progesterone to Alter the Estrual Cycle. E. L. WILLETT, *American Foundation for the Study of Genetics*
- P27 A Preliminary Report on the Role of Progesterone in the Maintenance of Pregnancy in the Cow. JAMES I. RAESIDE AND C. W. TURNER, *Missouri Agricultural Experiment Station*



- P28 Relative Reactions of European and Indian Cattle to Changes in Environmental Temperature. S. BRODY, H. H. KIBLER, A. C. RAGSDALE, *Missouri Agricultural Experiment Station*; H. J. THOMPSON, *U.S.D.A.*
- P29 A Study of the Effect of Two and Three Times a Day Milking Upon Milk Yield. J. G. CASH AND W. W. YAPP, *University of Illinois*
- P30 Observations of the Rate of Milk Removal. K. E. HARSEBARGER, *University of Illinois*
- P31 The Utilization of Acetic Acid by the Perfused Mammary Gland. G. L. MCCLYMONT AND J. C. SHAW, *University of Maryland*
- P32 Variations in Residual Milk Obtainable by Oxytocin Injections. ERIC W. SWANSON AND S. A. HINTON, *University of Tennessee*
- P33 Evaluation of Mammary Gland Development of Heifer Calves. J. H. BOOK, W. W. SWETT AND C. A. MATTHEWS, *Bureau of Dairy Industry, U.S.D.A.*
- P34 Effects of Udder Innunction with Diethylstilbestrol on Mammary Congestion in First-calf Heifers. JOSEPH MEITES, R. E. HORWOOD, E. P. REINEKE, C. S. BRYAN AND E. S. SMILEY, *Michigan Agricultural Experiment Station*
- P35 Estimation of the Thyroxine Secretion Rate without Sacrifice of the Animals. G. W. PIPES, C. R. BLINCOE AND KUANG-MEI HSIEH, *Missouri Agricultural Experiment Station*
- P36 Persistence of Different Causative Organisms in Mastitis Infections. LLOYD A. BURKEY, CECILIA R. BUCKNER AND W. W. SWETT, *Bureau of Dairy Industry, U.S.D.A.*
- P37 Factors Involved in the Whiteside Reaction. W. E. PETERSEN, J. F. GRIMMELL AND I. A. SCHIPPER, *University of Minnesota*

9:00-12:00

Section B. **NUTRITION AND PHYSIOLOGY.** L. O. GILMORE, *Chairman*

*Room 25, Warren Hall*

- P38 The Influence of Soybean Hay on Reproduction in the Rabbit. K. A. KENDALL AND G. W. SALISBURY, *University of Illinois*
- P39 A Duodenal Fistula for Physiological Studies in the Bovine. G. M. WARD, F. W. YOUNG AND C. F. HUFFMAN, *Michigan State College*
- P40 Tests with Sulphur Dioxide for Forage Preservation. J. B. SHEPHERD, H. G. WISEMAN, R. E. ELY, C. G.

MELIN, C. H. GORDON; L. G. SCHOENLEBER AND L. E. CAMPBELL; W. H. HOSTERMAN, *Bureau of Dairy Industry, Bureau of Plant Industry, Soils and Agriculture Engineering, and Production and Marketing Administration, U.S.D.A.*

- P41 A Comparison of Fecal Nitrogen Excretion Rate, Chromium Oxide and "Chromogen (s)" Methods for Evaluating Forages and Roughages. P. G. WOOLFOLK, C. R. RICHARDS, R. W. KAUFMANN, C. M. MARTIN AND J. T. REID, *Cornell University*
- P42 A Study of the Use of Chromium Oxide and Lignin as Indicators of Digestibility. E. A. KANE, W. C. JACOBSON AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*
- P43 The Effect of Dosage Level and Method of Administration of DDT on the Concentration of DDT in Milk. R. E. ELY AND L. A. MOORE, R. H. CARTER, H. D. MANN AND F. W. POOS, *Bureau of Dairy Industry, Bureau of Entomology and Plant Quarantine, U.S.D.A.*
- P44 Studies on Casein Utilization by Young Calves by Use of Radioactive Tracers. G. P. LOFGREEN, *University of California*
- P45 The Biological Activity of Alpha and Beta Casein-thyroprotein. M. B. BEHRENS, G. W. PIPES AND C. W. TURNER, *Missouri Agricultural Experiment Station*
- P46 Thyroxine Content of Synthetic Thyroprotein as Determined by a Radioactive Isotope Dilution Technique. E. P. REINEKE, D. P. WALLACH AND L. P. WOLTERINK, *Michigan State College*
- P47 The Influence of Thyroprotein Feeding on Gains in Body Weight of Dairy Calves. RALPH P. REECE, *New Jersey Agricultural Experiment Station*
- P48 The Use of X-Rays for the Detection of Rickets in Calves. J. W. THOMAS, *Bureau of Dairy Industry, U.S.D.A.*
- P49 An Approach to the Problem of the Etiology of Ketosis in Dairy Cows. B. C. HATZIOLOS AND J. C. SHAW, *University of Maryland*

Wednesday, June 21

Afternoon Session. *Bailey Hall*

1:30- 4:00

# JOINT MEETING OF PRODUCTION AND EXTENSION SECTIONS

G. M. CAIRNS AND C. W. REAVES, *Co-chairman*

### Symposium—Grassland Utilization and its Relation to Dairying

1. The Significance of Grassland Farming in the Dairy Economy. F. B. MORRISON, *Cornell University*
2. The Composition and Conservation of Forages. L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*
3. Pastures and the Dairyman. V. G. SPRAGUE, *Northeastern Regional Pasture Laboratory*
4. The Extension Dairyman and Grassland Farming. GEORGE WERNER, *University of Wisconsin*

4:00–5:00

### JOINT COMMITTEE REPORTS

The Program of the Purebred Dairy Cattle Association.  
FRED IDTSE, *Secretary, Purebred Dairy Cattle Association*

Breeds Relations. A. R. PORTER, *Chairman*

Dairy Cattle Health. JOE NAGEOTTE, *Chairman*

Dairy Cattle Breeding. J. S. TAYLOR, *Chairman*

Type Classification. L. O. GILMORE, *Chairman*

Thursday, June 22

Morning Session

9:00–11:00

Section A. **FEEDING.** G. M. CAIRNS, *Chairman*  
*Room 100, Caldwell Hall*

- P50 Kentucky 31 Fescue as a Dairy Pasture in Northern Ohio. AVERY D. PRATT AND JAMES L. HAYNES, *Ohio Agricultural Experiment Station*
- P51 Fourteen Years with Supplementary Pastures. N. R. THOMPSON AND C. W. HOLDAWAY, *Virginia Agricultural Experiment Station*
- P52 The Effects of Partial Replacement of Alfalfa Hay with Concentrate on Milk Production. K. A. KENDALL AND R. D. ENGBERSON, *University of Illinois*
- P53 Efficiency of Silage and Extra Grain Feeding for Maintaining Summer Milk Production. DWIGHT M. SEATH AND RALPH F. ELLIOTT, *Kentucky Agricultural Experiment Station*
- P54 Rates of Grain Feeding for Dairy Heifers on Temporary and Permanent Winter Pasture. S. H. MORRISON AND J. F. DEAL, *University of Georgia*
- P55 Kudzu and Fescue-ladino Clover Silage for Dairy Cows. W. A. KING AND J. P. LAMASTER, *South Carolina Agricultural Experiment Station*
- P56 Farm Grains vs. a Medium Protein Concentrate Mixture for Cows. K. E. GARDNER, *University of Illinois*

- P57 The Relative Palatability of Expeller and Extracted Linseed Meal in Dairy Cow Rations. N. N. ALLEN, *University of Wisconsin*

9:00-11:00

Section B. **REPRODUCTION.** L. O. GILMORE, *Chairman*  
*Room 25, Warren Hall*

- P58 Preliminary Observation on the Effects of Nutrition on the Quality and Quantity of Bovine Semen. H. H. OLSON, W. E. PETERSEN, T. W. GULLICKSON AND J. N. CUMMINGS, *University of Minnesota*
- P59 A Preliminary Report on the Effect of the Site of Semen Deposition on Fertility in Artificial Insemination. G. W. SALISBURY AND N. L. VANDEMARK, *University of Illinois*
- P60 Spermatozoan Transport in the Reproductive Tract of the Cow. N. L. VANDEMARK AND A. N. MOELLER, *University of Illinois*
- P61 The Effect of Sterile Copulation on the Time of Ovulation in Dairy Heifers. GERMAIN B. MARION, VEARL R. SMITH, THOMAS E. WILEY AND GEORGE R. BARRETT, *University of Wisconsin*
- P62 Measuring Reproductive Efficiency in Dairy Cattle. F. A. BUSCHNER, R. E. JOHNSON, C. I. BLISS AND A. A. SPIELMAN, *University of Connecticut*
- P63 The Sex Ratio in Calves Resulting from Artificial Insemination. K. E. GARDNER, *University of Illinois*
- P64 Artificial Breeding in Alaska and the Effect of Extra Light During the Short Winter Days. WILLIAM J. SWEETMAN, *Alaska Experiment Station*

11:00-12:00

**PRODUCTION SECTION BUSINESS MEETING,**  
*Room 25, Warren Hall*

*Thursday, June 22*

Afternoon Session

1:30- 3:00

Section A. **MANAGEMENT.** G. M. CAIRNS, *Chairman*  
*Room 100, Caldwell Hall*

- P65 Nutritive Value of Crops and Cows' Milk as Affected by Soil Fertility. II. The Amino Acid Composition of Colostra and Milk. C. W. DUNCAN, K. M. DUNN AND GERTRUDE I. WATSON, *Michigan Agricultural Experiment Station*
- P66 Dairy Cow Stall Studies. I. D. PORTERFIELD, GEORGE HYATT, JR., D. P. BROWN AND A. D. LONGHOUSE, *West Virginia University*

- P67 Calf Losses from Disease. H. P. DAVIS, *University of Nebraska*
- P68 Relation of Production Records of Cows and Efficiency Management of the Dairy Farm. LEO R. FRYMAN, *University of Illinois*
- P69 Relation of Gestation to Body Weights of Cows on Long-time Feeding Trials. R. B. BECKER, P. T. DIX ARNOLD AND SIDNEY P. MARSHALL, *Florida Agricultural Experiment Station*
- P70 The Influence of Oestrus on Weights of Holstein and Jersey Heifers. H. B. MORRISON, *Kentucky Agricultural Experiment Station*

1:30- 3:00

Section B. **SEMEN TECHNIQUES.** L. O. GILMORE, *Chairman*

*Room 25, Warren Hall*

- P71 The Effect of a Combination of Penicillin, Streptomycin and Sulfanilamide upon the Fertility of Bull Semen. J. O. ALMQUIST AND P. W. PRINCE, *Pennsylvania State College*
- P72 Bull Semen Toxicity of Various Salts, Brands and Lots of Penicillin, Streptomycin, Aureomycin and Chloromycetin. JAMES G. SYKES AND JOHN P. MIXNER, *New Jersey Agricultural Experiment Station*
- P73 The Influence of Aureomycin upon the Livability and Bacterial Content of Bull Semen. R. M. MYERS, J. O. ALMQUIST AND P. W. PRINCE, *Pennsylvania State College*
- P74 Hyaluronidase and Fertility of Dairy Bull Semen. JAMES E. JOHNSTON AND JOHN P. MIXNER, *New Jersey Agricultural Experiment Station*
- P75 A Comparison of Two Streptomycin Compounds Used in Diluted Bull Semen. H. L. EASTERBROOKS, P. HELLER, W. N. PLASTRIDGE, E. L. JUNGHER AND F. I. ELLIOTT, *University of Connecticut*
- P76 Results of Breeding Dairy Cows with Egg Yolk Citrate and Ortho Semen Diluters. VICTOR HURST, *South Carolina Agricultural Experiment Station*

#### PROGRAM OF EXTENSION SECTION

*Tuesday, June 20*

Afternoon Session. *Room 125, Warren Hall*

1:30- 4:30

**OPENING BUSINESS SESSION AND TEACHING METHODS AND EXHIBITS.** C. W. REAVES, *Chairman*

Address: Effects of Extension in New York. L. R. SIMONS, *Director of Extension, Cornell University*

E1 Master Package for Integrated County Meetings on Dairy Farm Management. S. N. GAUNT, *Massachusetts State College*

E2 Dairy Sub-committee Work in the County Agricultural Planning Program. A. LEON HOLLEY, *University of Arkansas*

Report of Teaching Methods Committee

Explanation and Discussion of Exhibits. JOHN W. FOSTER, *University of Kentucky*, in charge

(Exhibits in Room 101, Warren Hall)

Wednesday, June 21

Morning Session. Room 125, Warren Hall

9:00-12:00

**DAIRY HERD IMPROVEMENT ASSOCIATIONS AND DAIRY CATTLE BREEDING.** RAY ALBRECTION, *Chairman*

E3 Symposium on DHIA Organization, Operation and Record Analysis by Dairy Records Committee. R. G. CONNELLY, *Committee Chairman*

A. Selection, Training and Orientation of DHIA Personnel. C. R. GEARHART, *Pennsylvania State College*, AND ASSOCIATES

B. DHIA Record Analyses—

*Month to Month Analyses.* D. L. VOELKER, *Iowa State College*, AND ASSOCIATES

*Annual Analyses.* J. F. KENDRICK, *Bureau of Dairy Industry*; L. H. STINNETT, *Oklahoma A. and M. College*, AND ASSOCIATES

C. Organization and Operation of DHIA's. J. D. BURKE, *Cornell University*, AND ASSOCIATES

E4 A Comparison of Monthly, Bi-monthly, and Quarterly Tests for Estimating Milk and Butterfat Production of Dairy Cattle. J. E. STALLARD, *University of Wisconsin*

Report of Dairy Records Committee

E5. The Nature of Reproductive Failures in Dairy Cattle. Illustrated. T. Y. TANABE, *Pennsylvania State College*

*Wednesday, June 21*

Afternoon Session. *Bailey Hall*

1:30- 4:00 **JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS AND JOINT COMMITTEE REPORTS.** (See Production Section Program)

4:00- 5:00 **COMMITTEE AND BUSINESS MEETINGS**

*Thursday, June 22*

Morning Session. *Room 125, Warren Hall*

9:00-12:00 **4-H CLUB WORK, COMMITTEE REPORTS, BUSINESS MEETING.**

C. W. REAVES, *Chairman*

E6 Methods Used in Promoting the Junior Dairy Project.  
GLEN W. VERGERONT, *University of Wisconsin*

E7 How the American Dairy Science Association and the  
U. S. Department of Agriculture Can Aid in the De-  
velopment of 4-H Dairy Club Plans and Program.  
E. W. AITON

E8 Present Dairy Achievement Day and Dairy 4-H Show  
Ring Activities and How They Can Be Improved.  
L. A. HIGGINS, *Mississippi*

E9 Judging Contests, Their Development and Limitations.  
H. R. SEARLES, *Minnesota*

E10 Exhibit of Materials Used in Training 4-H Club Mem-  
bers and 4-H Club Leaders. RALPH PORTERFIELD,  
*Maryland*

Report of 4-H Club Committee. M. J. REGAN, *Com-  
mittee Chairman*

**COMMITTEE REPORTS**

**BUSINESS MEETING**

*Thursday, June 22*

Afternoon Session. *Room 125, Warren Hall*

1:30- 3:00 **DAIRY CATTLE HEALTH.** C. W. REAVES, *Chairman*

E11 Hyperkeratosis. PETER OLAFSON, *Cornell University*

E12 Methods of Conducting an Educational Program on  
Area Brucellosis Control. E. C. SCHEIDENHELM, *Rut-  
gers University*

E13 Dairy Stable Ventilation for Mastitis Prevention.  
JOSEPH C. NAGEOTTE, *Pennsylvania State College*

E14 Bacteria in the Cow's Udder Associated With Mastitis.  
JAMES J. REID, *Pennsylvania State College*

E15 Control of Coccidiosis in Dairy Cattle. READ L.  
DAVIS, *Federal Regional Disease Laboratory, Alabama*

## JOURNAL OF DAIRY SCIENCE

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ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-FIFTH  
ANNUAL MEETING

## PRODUCTION SECTION

**P1. A study of the inheritance of persistency in milk production.** M. H. ALEXANDER, Univ. of Illinois

The data collected in this study show that, (a) the daughters of inbred sires vary less in their average persistency values ( $P$  values) than the daughters of non-inbred sires; (b) inbred daughters of each sire studied varied less in their  $P$  values than the non-inbred daughters of the same sire; (c) there is a distinct difference in the average  $P$  values of the cow groups by breeds; (d) there is a difference in the average  $P$  values between strains within the breeds; (e) there is a difference in the average  $P$  values of the daughter groups of different sires; (f) there is less variation in the  $P$  values of repeat pairs than of random pairs under the same environment; and (g) the regression of the  $P$  value of daughters on dams is consistent. From the results obtained in the analysis of these data it is concluded that persistency of lactation in dairy cows is a heritable factor.

**P2. Some of the effects of calving interval on milk and butterfat production of Ayrshire cattle.** W. J. TYLER AND GEORGE HYATT, JR., West Virginia University

The records used in this study were two-time M.E., 305 d. or less lactations taken from punch cards furnished by the Ayrshire Breeders' Association. There were a total of 2,203 animals studied. The average length of lactation was obtained by averaging the number of days in milk, with no cow being allowed more than 305 d. for a single lactation.

The data indicate that milk and butterfat production of cows with 10- or 11-mo. calving intervals is significantly lower than those having 12- or 13-mo. intervals. However, it is apparent that this lower production is due in part to a shorter average lactation. Study of the 2nd and 3rd lactations indicates that these cows with short calving intervals may be persistently poorer producers. The data also indicate that the unfavorable influence of a short calving interval may persist into the succeeding lactation. Significantly

greater production was not obtained when cows had 14- or 15-mo. or longer calving intervals as compared with the 12- or 13-mo. intervals. The correlation between first and second calving intervals was found to be +0.10.

**P3. A study of the type ratings of daughters of sires and dams that have been classified for type.** W. J. TYLER, West Virginia University

A previous study of official type ratings of Ayrshire cows indicated that the heritability of type was approximately 0.3. However, no estimate of the inheritance of the sire's type was made. Since some breeders of dairy cattle select for type on the basis of the type ratings of bulls and cows, it should be important to know the results of matings between sires and dams with the different classification ratings.

The first official and the highest official type rating of 2,005 daughters that were by 189 classified sires and out of classified dams have been studied to determine the probable effectiveness of selection for type in dairy cattle. The average rating of the daughters out of various combinations of classified sires and dams compared favorably with expected ratings computed from the heritability estimate of 0.3 and the difference between the parents and the average type ratings of the breed. These breed averages were 83.0 (first official rating) and 83.7 (highest official rating) for cows and 84.8 for bulls (based on Excellent = 92.5; Very Good = 87.5; Good Plus = 82.5; Good = 77.5; Fair = 72.5).

The results, when the highest official type rating was used, are summarized as follows: Excellent bulls mated to cows averaging 85.2 for type had daughters averaging 84.6; very good bulls mated to cows averaging 84.5 for type had daughters averaging 84.0; good plus bulls mated to cows averaging 85.0 for type had daughters averaging 83.4; good bulls mated to cows averaging 84.4 for type had daughters averaging 81.7. Use of the first official rating on each of the animals gave similar results.

**P4 Preliminary report on the production records of crossbred dairy cattle.** J. P. LAMASTER,



G. W. BRANDT AND C. C. BRANNON, S. C. Agr. Expt. Sta., AND M. H. FOHRMAN, Bureau of Dairy Industry, U.S.D.A.

The cross-breeding of dairy cattle was started at this station in 1936. Two-breed females were produced by mating animals of different breeds. These two-breed females then were crossed with sires of a third breed to produce three-breed females. In a similar manner four- and five-breed females have been produced.

The crossbred group is handled in the same manner as the purebred groups in the station herd. They receive all the roughage they will consume and a concentrate mixture at the rate of 1 lb. of grain to 3 lb. of milk (4% F.C.M.). Production records are based on daily milk weights and monthly butterfat tests, for 305-day lactations. In December, 1941, all cows were changed from 3x to 2x milking. Production comparisons will be given for 13 two-breed crossbreds and their purebred dams; 7 two-breed females and their 13 purebred maternal sisters; 13 three-breed and 8 four-breed crossbreds and their dams. All sires, except one, have been proved in the station herd on purebred daughters. These proofs will be used in giving the results obtained to date.

**P5. Inheritance of butterfat test in the Beltsville Holstein herd.** JOSEPH B. PARKER AND CHARLES A. MATTHEWS, U.S.D.A.

Butterfat tests were available on 361 Holstein cows in the Beltsville breeding herd. No selection has been practiced and all were tested for production on a standardized feeding and management basis. Correlation, analysis of variance and covariance have been used in an attempt to determine the inheritance of butterfat test. A highly significant correlation of 0.4850 was found with 316 dam-daughter pairs with a regression equation of  $Y = 1.8564 + 0.5199X$ . Analysis of variance shows a highly significant difference between the sires used. Allowing for the different butterfat test levels of the sires' mates, covariance shows highly significant differences between sires. Sorting by generations, where the use of sires overlap, gave highly significant differences by analysis of both variance and covariance. Cow-families show no significant differences. Postulating on heterozygous make-up with a minimum of 4 pairs of gene test factors, it is possible to approximate the actual results. Culling for low test would have increased the average test by only 0.04% and cut the numbers in half. On the basis of the present herd of 59 cows with completed records, there would only have been 33 cows testing 3.89%. The test of the 26 cows eliminated averaged 3.89%.

**P6. A selection index for fat production utilizing the fat yields of the cow and her relatives.** J. E. LEGATES AND JAY L. LUSH. Iowa State College

The object of this study was to devise an index for selecting dairy cattle more accurately by utilizing the fat yields of the individual and its relatives. Relatives considered were the cow's dam, daughters, maternal half sisters and paternal half sisters. Information about the fat yields of the cow and her relatives was combined in such a way that the index value for an animal was more highly correlated with its breeding value than if the information were combined in any other linear manner. Statistics needed to construct the index were calculated on an intra-herd basis; thus, the index is for selecting between individuals kept under the same general herd environment. These statistics were computed from an analysis of 23,330 lactation records from 12,405 cows in 293 Jersey herds on H.I.R. test during 1943 to 1947.

For heritability of observed differences in fat yield of 0.20, as determined from these data, information on the individual should receive about 2.75 times as much attention as the same information on the dam. Progress to be expected by using the index for selections between cows with records of their own would be about 1.10 to 1.15 times faster than by making selections on own performance alone.

**P7. An index for the effects of certain environmental influences on dairy cattle production.** N. D. BAYLEY AND E. E. HEIZER, Univ. of Wisconsin

This study is concerned with evaluation of the effects of specific environmental influences on sire provings. The data consist of information obtained on 967 cows in 47 different Holstein-Friesian herds of Wisconsin. The influences considered are condition before freshening as a heifer, age at first freshening, length of dry period, condition during dry period, age at last freshening, days with calf during lactation, length of lactation, lb. TDN daily/1000 lb. body weight, nutritive ratio of ration during barn feeding period of lactation and selection and number of milking cows in the herd. Ratings and scoring systems have been devised for influences not readily evaluated numerically.

Multiple regression techniques have been used to estimate the importance of the environmental factors and also the success of the devised measuring systems. The effects of the important factors have been combined in an index which expresses environmental variation in terms of butterfat. Further investigations concerning the use and validity of this index are discussed.

**P8. Heritability of fertility in dairy cattle.** R. S. DUNBAR, JR., AND C. R. HENDERSON, Cornell Univ.

Reduced fertility in dairy cattle is a serious economic problem, but little is known concerning the effectiveness of selection for high fertility and what relative emphasis in selection should be placed on fertility and on other important economic traits. Quantitative measures of the heritability of fertility and of the phenotypic and genetic correlations between fertility and other important traits are needed to answer these questions. Records of breeding performance and production of artificially sired cows offer an unusual opportunity to study these factors, as well as the joint effect of genetic "nicking" and genetic-environmental interaction.

Two measures of fertility, non-returns to 1st service and calving interval, have been employed in this study. By means of an estimation procedure which yields unbiased estimates of components of variance in non-orthogonal data, estimates were obtained of the variance due to additive genetic differences among sires. Sire variance was essentially zero in both studies. Therefore, heritability of fertility is estimated to be near zero.

From these results it is concluded that under the present conditions and using non-returns or calving interval as a measure of reproductive efficiency, selection for fertility in dairy cattle must be quite ineffective. Consequently, emphasis in selection should be placed on other traits of economic importance for which selection is known to be effective.

**P9. Predicting the breeding efficiency of dairy cows.** DURWARD OLDS AND D. M. SEATH, Kentucky Agr. Expt. Station

Data were tabulated from the herd record books of 20 local cooperative inseminating units of the Kentucky Artificial Breeding Association. Included in the study were 6,509 cows and 2,403 herds which were serviced both of 2 consecutive yr. It was found that 4,665 cows, each of which required only 1 service the 1st yr. averaged 1.44 services the 2nd yr.; 1,372 cows requiring 2 services the 1st yr. averaged 1.54 services the 2nd yr.; 400 cows requiring 3 services the 1st yr. averaged 1.64 services the 2nd yr. The 72 cows which required 4 services the 1st yr. averaged 1.65 services the 2nd yr.

The correlation between breeding efficiency for consecutive years was  $0.084 \pm 0.012$ . This correlation, though statistically highly significant, was too small to indicate a high degree of predictability.

The predictability of breeding efficiency of

herds as units was about the same as that for cows. Only 9.3% of the "problem herds" (averaging 2.1 or more services/cow) were still problem herds the next year. The total number of problem herds was 7.1% the 1st yr. and 5.9% the 2nd yr. The average herd consisted of 7.1 cows the 1st yr. and 8.3 cows the 2nd yr.

**P10. The frequency distribution of cellular antigens in five breeds of dairy cattle.** L. C. FERGUSON, F. J. LAZEAR AND FORDYCE ELY, Ohio State Univ.

The recognition of more than 40 cellular antigens in the blood of cattle has contributed a tool for an analytical study of inherited characters. A study of the distribution of the antigens in the different breeds reveals additional evidence to support the idea proposed by Lush that breed differences are dependent on variations in the gene frequency in a given population. The paper of Owen, *et al.* (J. Animal Sci., 3:315, 1944) reported the observations on the frequency distribution of the cellular antigens in 2 breeds of dairy cattle. This report essentially confirms those observations and extends them to the other dairy breeds.

The results reported are based upon the test of 1,205 Holstein, 1,025 Guernsey, 443 Jersey, 211 Brown Swiss and 80 Ayrshire cattle with serum reagents for 36 to 40 different antigens. The data indicate some marked differences in the occurrence of certain antigens in the 5 breeds. Generally, the distribution of antigens is quite similar in the Jersey and Guernsey cattle tested. In the same way, there is a resemblance in the components of Holstein and Ayrshire cattle. There is a tendency for the frequency of the antigens in the Brown Swiss cattle to place this breed between the Guernsey and Jersey on the one side and the Holstein and Ayrshire on the other.

**P11. Length of gestation in the five major breeds of dairy cattle.** M. H. ALEXANDER, Univ. of Illinois

The Brown Swiss and Guernsey were shown to have a distinctly longer gestation period than the generally accepted 281-day average, while the Ayrshire, Holstein-Friesian and Jersey breeds were distinctly below this average. For all the breeds the gestation period for twins was less than for singles. Gestation periods preceding calvings in the summer months were somewhat shorter than for other seasons.

There is a slightly positive, but statistically insignificant, effect of increasing length of gestation on the following items which might affect production of milk and fat: (a) length of gestation

on persistency of secretion of milk and fat; (b) length of gestation on length of lactation; and (c) length of gestation on production of FCM.

The study shows conclusively that length of gestation is a heritable factor, since there is a distinct difference by breeds and since the grouped offspring of different sires within each breed show marked differences in time spent *in utero*.

**P12. The preparation of a riboflavin-deficient milk for experimental calf feeding.** E. G. MOODY, S. M. HAUGE AND N. S. LUNDQUIST, Purdue Univ.

Irradiation of skim milk with ultra-violet light yielded a product averaging 0.12 mg. riboflavin/l. (94% destruction) as determined by microbiological assay. Three gal. of milk in a glass jar, while being stirred, were treated with a 600 w. quartz mercury vapor lamp at a distance of 9 in. Five hr. irradiation at 60-65° C. produced the same destruction as 12 hr. at room temperature. Two infra-red and 2 flood lamps were used to supply heat but apparently had no photolyzing effect.

By means of chromatographic adsorption technique, using Florisil as the adsorbent, 98% of the riboflavin was removed from either skim or whole milk. This was accomplished in about 1/3rd the time required for irradiation and apparently did not change the flavor. Five gal. of milk were drawn with the aid of suction through a 3-in. column containing 1 lb. of Florisil. Whole milk required heating to about 45° C. The filtrate contained about 0.034 mg. riboflavin/l. as determined photofluorometrically.

**P13. The influence of rumen inoculations on the digestibility of dry matter, cellulose and protein in young dairy calves.** H. R. CONRAD, J. W. HIBBS, W. D. POUNDEN AND T. S. SUTTON, Ohio Agr. Expt. Station

Thirty-two digestion trials were conducted to determine if calves inoculated with cud material from older cattle and fed rations high in roughages would be able to digest dry matter, cellulose and protein more efficiently than uninoculated calves.

Five inoculated and 5 uninoculated calves first were fed a ration of whole milk and alfalfa hay *ad libitum* for 14 d. During this period, the calves which were inoculated digested a significantly higher percentage of ingested cellulose (difference 4.81%) and dry matter (difference 3.84%) than the calves which were uninoculated. However, this appreciable difference in digestibility between inoculated and uninoculated calves disappeared when some of these calves later were placed on rations of either alfalfa hay or grass clippings only. No differences were found

in the apparent digestibility of protein on any of the rations.

A calf which previously had been freed of characteristic "indicator" rumen microorganisms by heavy grain feeding showed a marked decreased ability (11.26%) to digest cellulose when placed on an alfalfa hay ration, in contrast to a similarly treated calf in which the characteristic microorganisms had reappeared, possibly through natural reinoculation.

**P14. Variations in digestibility of certain characteristic rumen microorganisms and some effects of their absence on calves.** W. D. POUNDEN AND J. W. HIBBS, Ohio Agr. Expt. Station

Contents of various parts of the digestive tract of cattle were examined for the presence of usual varieties of rumen microfauna and 4 species of microflora which characteristically were present in their rumens. The 4 microflora types were larger coccoids, large cigar-shaped rods, small rods in flat rectangular groups and large thick square-ended rods.

The disappearance of usual rumen protozoa and the large cigar-shaped rods from abomasal and intestinal contents, as reported by others, was confirmed in this study. Some of the larger coccoids were observed in all parts of the digestive tract. The small rods and the large thick rods appeared gradually to disintegrate as they reached posterior parts of the tract. The ultimate fate of rumen microorganisms thus appears to vary between the extremes of complete destruction in the abomasum to passage entirely through the tract.

A Jersey calf was raised to 6 mo. of age with a rumen free since birth of usual rumen protozoa but containing massive numbers of the large cigar-shaped digestible rod. It had a neat and healthy appearance like 12 similar calves which received rumen inoculations and which were fed similar feeds. Three other calves whose rumens were devoid of both usual protozoa and the 3 rods but which contained the larger coccoids had rough hair coats and their abdomens appeared abnormally deep and pot-bellied.

**P15. Some chemical and physical characteristics of the contents of the alimentary tract of calves at time of birth.** D. B. PARRISH AND F. C. FOUNTAINE, Kansas Agr. Expt. Station

At time of birth of the calf all sections of the alimentary tract contain a mixture of fluids and various solids, including hair. Total weight of alimentary contents obtained from 5 calves ranged from 609-958 g.; dry matter from three calves, 113-168 g. Average pH of the contents decreased in the following order: stomach, cae-

cum, small intestine, large intestine and colon. In general, the nearer the anus the contents were found, the greater the percentage of dry matter and the darker the color. Dry matter of stomach contents was 1.4% and of colon contents 26.5%. Average percentage of various substances found in the meconium from the large intestine was crude protein, 11.5; ether extract, 2.9; ash, 1.1; and N.F.E., 10.1. Ions identified in the ash of the meconium were chlorides, sulfate, carbonate, phosphate, silica, sodium, potassium, calcium and magnesium. Results of other analyses will be presented.

**P16. Postpartum development of bovine stomach compartments and observations on some characteristics of their contents.** SIDNEY P. MARSHALL, P. T. DIX ARNOLD AND R. B. BECKER, Univ. of Florida.

Jersey male calves have been anesthetized, the thoracic and abdominal cavities opened, apertures to the rumen, reticulum, omasum and abomasum closed by clamps and the stomach removed. Volume displacement of each compartment and of tissue of each compartment were measured, tissue and contents of each compartment weighed and pH and specific gravity determined where adequate quantities of materials were available.

Weight and volume of rumen tissue and weight of fresh contents exceeded that of the abomasum between 7 and 30 d. of age. Omasum and reticulum development also proceeds rapidly in young calves. Aliquots of stomach compartment contents taken in open containers for pH analysis ranged as follows: rumen, 5.17-6.89; reticulum, 5.19-7.19; omasum, 4.98-6.81; and abomasum, 2.1-4.61.

Consumption of grass, leaves, hay, excelsior bedding and sand occurred in increasing amounts during early life, as indicated by their presence in the stomach compartments.

**P17. The growth of dairy calves on purified diets.** G. P. LOFGREEN, Univ. of California

The response of male dairy calves to two purified diets has been determined. The diets differed in their mineral composition and the methods used to dissolve the casein and to emulsify the fat.

In one diet the casein was dissolved in  $\text{NaHCO}_3$ , the fat was homogenized into the mixture and the entire mineral mixture added at one time. Milk prepared in this manner always contained a heavy precipitate of minerals. Calves fed this diet grew at a subnormal rate, scoured more frequently than the control calves and frequently showed loss of hair.

The second diet was made by dissolving the casein in  $\text{Ca}(\text{OH})_2$ , adding the minerals in steps from various mineral solutions and emulsifying the fat by use of lecithin or commercial non-ionic emulsifying agents. This milk had no precipitate, thus facilitating the feeding on an homogeneous mixture. Calves fed this diet scoured less, showed less loss of hair, but gained at a subnormal rate.

In both groups the critical period appeared to be the first 3 wk. During this period, calves either lost weight or merely maintained their weight. From 3-8 wk. the gains approached normal.

**P18. The nutritive value of starch and the effect of lactose on the nutritive values of starch and corn syrup in synthetic milks for young calves.** R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN AND H. D. WEBSTER, Michigan Agr. Expt. Station

Nine neonatal calves, 6 Holstein, 2 Jersey and 1 Ayrshire, were allotted to 3 experimental groups and fed rations consisting of synthetic milks which varied only in the carbohydrate component. Calves were separated from their dams at 4-12 hr. after birth, placed in individual pens and started on the synthetic milk diet after fasting for 24 hr.

Calves receiving corn syrup plus lactose (KL) gained an average of 28.33 lb., those receiving starch plus lactose (SL) gained an average of 24.67 lb., while calves on starch (S) averaged only a 14.00-lb. gain in 31 d. KL and SL calves gained uniformly throughout the trial; S calves gained little or none the first 2 wk. but gained rapidly the latter half of the trial. The efficiency of feed utilization, expressed as pounds of gain per pound of dry matter consumed, was 0.487, 0.412 and 0.204 for the KL, SL and S groups, respectively. Diarrhea was much more common in the S group than in the other two groups.

Serial blood samples were collected before a test meal and at 0.25, 0.5, 1, 2, 4, 6 and 8 hr. after feeding and analyzed for blood sugar. The blood sugar level rose rapidly after the ingestion of glucose, lactose or corn syrup, with the maximum concentration at 4, 4 and 1 hr. after feeding, respectively. Following starch ingestion, there was no change in blood sugar the first 4 hr. and only a moderate increase at 6 and 8 hr.

**P19. Is A.P.F. of value in a calf starter for calves weaned from milk at an early age?** L. L. RUSOFF AND M. O. HAQ, Louisiana State Univ.

A 19.4% digestible protein calf starter containing additional thiamine, riboflavin, calcium pantothenate and niacin, but no animal protein,

was compared with a similar ration supplemented with A.P.F.<sup>1</sup> so that the ration contained 10 mg. of vitamin B<sub>12</sub> per ton. The A.P.F. did not appear to be of any value for calves weaned from whole milk at 28 d. of age, when gains in weight, height at withers and feed efficiency were compared at 63 d. of age. The decrease in growth rate which always occurs after weaning calves from milk at an early age was not prevented by the feeding of A.P.F.

This experiment is still in progress. Results at the completion of the experiment (90 d. of age) will be available at the time of the meeting.

<sup>1</sup> A.P.F. no. 3. Courtesy Merck & Co., Rahway, N. J.

**P20. APF supplements in milk replacements for dairy calves.<sup>1</sup>** J. B. WILLIAMS AND C. B. KNOTT, Pennsylvania Agr. Expt. Station

Fifteen male Holstein calves, 7 d. of age, were divided into 3 comparable groups on the basis of height at the withers, body weight and chest circumference. All calves were fed the basal replacement mixture composed of the following ingredients: dried skim milk, 50 lb.; dried whey, 10 lb.; dried distiller's corn solubles, 10 lb.; soluble blood flour, 10 lb.; ground oat groats, 5 lb.; cereose, 7.75 lb.; dried brewer's yeast, 4.9 lb.; irradiated yeast (9F), 0.10 lb.; stabilized vitamin A feed (2,220,000 U.S.P. units/lb.), 0.22 lb.; mineral mix, 0.042 lb.; and dicalcium phosphate, 2.5 lb. Group I was fed the basal replacement, group II—basal replacement plus 2.2% APF supplement (3–4 µg./g. B<sub>12</sub> equivalent) and group III—basal diet plus 0.3 per cent APF supplement (27.5 µg./g. B<sub>12</sub> equivalent). The calves also were fed a suitable calf starter and good quality mixed hay *ad libitum*. The rate of feeding of the replacement was the same for all calves. Under the conditions of this experiment, it does not appear that the supplementation of the milk replacement used with APF improved the rates of growth in Holstein bull calves.

<sup>1</sup> The cooperation of Merck & Co., Rahway, N. J. and Lederle Laboratories, Pearl River, N. Y. is acknowledged and appreciated in the conduct of this trial.

**P21. Serum protein fractions of calf blood as influenced by colostrum and skim milk.** R. F. ELLIOTT AND CECIL CONLEY, Kentucky Agr. Expt. Station

Whole colostrum or colostrum cream in skim milk was fed to each of 9 calves for 2 d. The calves were bled periodically and the serum pro-

tein fractions estimated colorimetrically after the method of Wolfson *et al.* (Am. J. Clin. Path., 18: 723, 1948). At birth, the average values found for total protein were 4.0 g./100 ml. serum and 2.37, 1.66, 0.68, 0.82 and 0.20 for albumin, total, alpha, beta and gamma globulins, respectively. Following the consumption of colostrum, immediate increases of the various fractions were observed with maximum values being reached within 20–30 hr. after birth. The various fractions then leveled off with some fluctuations and with no tendency to decrease. Calves which were fed the skim milk did not show immediate increases in serum protein fractions and only a slight tendency to increase to 10 d. of age. The gamma globulin fractions for these calves were extremely low. As compared to normal calves, the average total serum protein of new born calves was two-thirds as great, while the average globulin fractions were only half as great as those observed for calves 2 mo. of age. Calves fed milk from their prepartum-milked dams follow the same pattern in respect to serum protein fractions as those observed for the skim milk-fed calves.

**P22. The effect of kind of carrier and method of dispersion on the absorption of carotene by young dairy calves.** J. W. CROWLEY AND N. N. ALLEN, Univ. of Wisconsin

Dairy calves, fed from birth on a carotene-free ration of goat colostrum and goat milk, were used to study the absorption of carotene. Their blood carotene was very low, and by partially substituting skimmed goats milk, the plasma vitamin A was kept at about 10 µg./100 ml.

For each treatment, a single feeding of 100 mg. of crystalline carotene was dispersed in 35 ml. of the oil being tested, which was fed to the calves in skimmed goats milk. Two methods of dispersion and 5 different oils were compared. Blood samples were collected before treatment, every 4 hr. after treatment for 24 hr. and every 24 hr. thereafter until the blood carotene had decreased to near the pre-treatment level.

Five carriers (corn, soybean, cow butter, goat butter and mineral oils) were used. When dispersion was accomplished by stirring, the average maximum carotene increases in 5 Guernsey calves for the 5 oils were 25, 26, 24, 28 and 8 µg./100 ml. of blood plasma, and for 5 Jerseys, 24, 24, 25, 32, and 7, respectively; when homogenization was used, Guernseys increased 65, 69, 69, 75 and 13, and the Jerseys increased 72, 69, 70, 80, and 14, respectively. The maximum increase was reached in 12–16 hr. and required 2–3 wk. to return to the pre-treatment level. There was no change in blood vitamin A follow-

ing treatment. Due to difficulties in making quantitative collections of feces, excretion data are not considered reliable, but in general, about two-thirds of the carotene fed was recovered in the feces for the 1st 4 oils and a greater amount for the mineral oil.

Much of the carotene appears to be absorbed as such and to linger in the blood of calves without being converted to vitamin A, even though the calves are at a low plane of vitamin A nutrition.

**P23. The riboflavin requirement of the very young calf.** GERMAIN J. BRISSON AND T. S. SUTTON, Ohio State Univ.

Nine dairy calves were used to determine the minimum riboflavin requirement for the calf up to 8 wk. of age. The calves were taken from their dams at birth and fed colostrum for 2 d., after which they were fed a photolyzed whole milk ration supplemented with vitamin A and with riboflavin at different levels. The riboflavin was supplemented at the levels of 0, 25, 35, 45 and 115  $\mu\text{g.}/\text{kg.}$  of body weight daily. The latter calf received the same amount of riboflavin as a similar calf fed an untreated whole milk diet. At the 0 and 25  $\mu\text{g.}$  levels, growth was reduced and deficiency symptoms appeared. At the 35  $\mu\text{g.}$  level, growth was apparently normal and practically all deficiency symptoms were eliminated. At the 45  $\mu\text{g.}$  level, performance of the calves was apparently normal. The average daily urinary excretion of riboflavin per 100 lb. of body weight was 0.06, 0.11, 0.12, 0.40, 2.66 and 2.29 mg. for the calves receiving 0, 25, 35, 45 and 115  $\mu\text{g.}$  of riboflavin/kg. and untreated milk, respectively. On the basis of these results, it is concluded that the minimum daily riboflavin requirement for the very young dairy calf is between 35 and 45  $\mu\text{g.}/\text{kg.}$  of body weight.

**P24. Synthesis of certain B-vitamins in the digestive tract of dairy calves.** E. M. KESLER AND C. B. KNOTT, Pennsylvania State College

Levels of thiamine, riboflavin and nicotinic acid were determined on the contents of the rumen, omasum, abomasum, small intestine and large intestine of male Holstein calves. In the 1st part of the experiment, 2 16-wk.-old calves were slaughtered at 2, 4, 6, 8 and 12 hr. after feeding, respectively. Concentrations of the 3 B-vitamins were found to be higher in all parts of the digestive tract than in the feed ingested on a dry matter basis. There was a decided peak in thiamine and nicotinic acid concentrations at 6 hr. after slaughter. Levels of riboflavin and nicotinic acid were much higher in samples from the small intestine than in other parts of the digestive tract.

In the second trial, 3 calves were slaughtered in each of the following age groups: 2, 4, 6, 8, 10, 12 and 14 wk. These calves were fed a maximum of 300 lb. of whole milk and calf starter up to 5 lb./d. with alfalfa hay *ad lib.* B-vitamin levels generally were higher on a dry matter basis in all parts of the digestive tract than in the feed given. This was true of all ages studied. Similarly, levels of riboflavin and nicotinic acid were higher in the small intestine than in the other regions studied. Much individual variation was noted throughout.

**P25. The effects of estrogen and progesterone on the arterial system of the uterus of the cow.** WILLIAM HANSEL, Cornell Univ.

The arterial systems of the uteri of 7 normal cows and 7 ovariectomized cows treated with varying amounts of estrogens and progesterone were studied histologically and by means of plastic injection-corrosion preparations. The arterial systems of the uteri of 16-mo.-old heifers and mature cows were found to differ markedly. The endometrial arterioles in the uteri of heifers were found to be essentially straight, while similar arterioles in the uteri of mature cows were found to be coiled on their central ends. Tightly coiled endometrial arterioles were found in the caruncles.

The degree of development of the arterial systems of the uteri of the ovariectomized cows was correlated with the amounts of the ovarian hormones administered during the 17-mo. period prior to the time they were slaughtered.

**P26. The fertility of heifers following administration of progesterone to alter the estrual cycle.** E. L. WILLETT, American Foundation for the Study of Genetics, Madison, Wis.

A study has been made to determine the fertility of yearling heifers inseminated artificially at the 1st heat following injection of progesterone to alter their estrual cycles. Fifty to 100 mg. of progesterone were injected daily starting on the 14th or 15th day of the cycle and continuing for 13-17 d. During estrus, each animal was bred when heat was first noted and again 24 hr. later. Fourteen heifers received 1 treatment and 4 received a 2nd when they failed to conceive the 1st time.

Estrus was observed, on the average, 5 d. following termination of injection (range: 4-7 d.). The resulting cycles varied from 28-34 d. in length. Eleven pregnancies were obtained from the 22 breedings. The inseminations for the 1st 13 breedings were performed by an inexperienced inseminator, and 5 or 39% resulted in pregnan-

cies. The last 9 were performed by an experienced inseminator, and 6 or 67% resulted in pregnancies. These results are within the range of breeding efficiency for heifers of this age as reported in the literature. The work is being continued.

**P27. A preliminary report on the role of progesterone in the maintenance of pregnancy in the cow.<sup>1</sup>** JAMES I. RAESIDE AND C. W. TURNER, Missouri Agr. Expt. Station

An attempt has been initiated to determine, quantitatively, the progesterone required for the maintenance of pregnancy in dairy heifers. Removal of the corpus luteum from 3 Holstein heifers in the early stages of pregnancy was performed by manual expression through a flank incision made after a positive diagnosis by rectal palpation. Daily subcutaneous injections of 25 mg. progesterone in olive oil were commenced 1 d. prior to the operation.

While the estimated ages of the fetuses at the time of the operation were 44, 48 and 76 d., abortion occurred in all 3 instances. By close observation it was possible to recover the fetuses at abortions 6, 16 and 12 d. after the respective corpus luteums had been removed. From this, it was concluded that 25 mg. progesterone daily was insufficient to maintain the pregnant condition at this stage. Increased amounts of progesterone will be tested until the pregnant condition can be maintained.

<sup>1</sup> Contribution from the Department of Dairy Husbandry, Missouri Agr. Expt. Station, Journal Series no. 1206.

**P28. Relative reactions of European and Indian cattle to changes in environmental temperature.<sup>1</sup>**

S. BRODY, H. H. KIBLER AND A. C. RAGSDALE, Missouri Agr. Expt. Station, and H. J. THOMPSON, U. S. D. A.

The zone of thermoneutrality for lactating cows under normal conditions is between 40 and 60° F. The increase in heat stress with increasing temperature up to 105° F. is much greater than the increase in cold stress with temperature decreasing to 0° F. and is much greater in European than Indian cows. Appreciable change with rising temperature occurred at the following temperatures: rectal temperature, 75° F. in European and 95° F. in Indian; pulse rate, 85° F. in European and 95° F. in Indian; respiration rate, 65° F. in European and 80° F. in Indian; hay consumption, 75° F. in European and 85° F. in Indian; the rate of change of these reactions was always steeper in European than Indian cows. Unlike in (sweating) man, surface

temperatures in European cows exceeds 95° F. when the environmental temperature exceeds 99° F. and the two meet at about 105° F. The water consumption above 85° F. increased in the Indian and decreased in European except Jersey 212. Gradually decreasing temperature from 50 to 5° F. did not affect the rectal temperature, but increased the heat production in Jersey and Holstein cows from 20 to 30%, more in small than large cows.

<sup>1</sup> Contribution from the Missouri Agricultural Experiment Station and the U. S. Dept. of Agr. (Mo. Sta., Journal Series 1203).

**P29. A study of the effect of two- and three-times-a-day milking upon milk yield.** J. G. CASH AND W. W. YAPP, University of Illinois

The chief objective of this experiment was to make a comparison between 2- and 3-times-a-day milking when environmental factors remained constant. After balancing the 2 sides of the udders as to yield, one-half of the udders were milked twice daily and the other 3 times. The experiment was conducted throughout complete lactation periods. Eight lactations on 7 different cows, consisting of 5 Holsteins, 1 Guernsey and 1 Ayrshire were studied. All cows used in the experiment were fed 3 times daily.

The total production of milk from the sides of the udders milked 3 times daily was 132% of the production from the sides milked twice daily. As lactation progressed, the percentage difference between the production of the halves of the udder milked 2 and 3 times daily became greater.

The average per cent fat content for the lactations was 3.68 for the milk from the sides of the udders milked 3 times daily and 3.63 for the milk from the sides milked 2 times daily.

**P30. Observations on the rate of milk removal.** K. E. HARSHBARGER, University of Illinois

The rate of milk removal during the milking process for individual cows milked in a milking parlor under standard conditions has been determined. The time between application of teat cups and initial flow and the cumulative amount of milk removed at intervals of 20 sec. were recorded. The rate of removal was calculated from an adjusted "end-point" of milking. The observations were taken on 2 d. of each month for the cows studied throughout a lactation period. Differences in the rate of removal between cows at various levels of production and within cows throughout the lactation have been evaluated.

The rate of milk removal increased with higher milk production and the total time re-

quired to milk high-producing cows was not proportionally higher than that of low-producing cows. The rate of removal for individual cows decreased throughout the lactation period along the same trend as the decrease in milk production.

**P31. The utilization of acetic acid by the perfused mammary gland.** G. L. McClymont and J. C. Shaw, Univ. of Maryland

The mammary glands of 4 cows were perfused with blood to which acetic acid was added. An artificial heart-lung was used to perfuse the glands. Acetic acid was determined on the half of the udder not perfused for a control and on the perfused half at the end of the perfusion. The blood was analyzed for acetic acid at the beginning and end of the perfusion. The utilization of the 4 glands in terms of 100 ml. of blood/100 g. of tissue was 0.17, 0.09, 0.10 and 0.16 mg., respectively.

**P32. Variations in residual milk obtainable by oxytocin injections.** ERIC W. SWANSON AND S. A. HINTON, Univ. of Tennessee

Residual milk was secured following a normal milking by injecting Pitocin intravenously and resuming milking. Ten cows were sampled at monthly intervals and 5 others were sampled only once at various stages of the lactation. The quantity of residual milk obtained bore a close relationship to the normal milking ( $r=0.43$ ). It was highest at the 2nd mo. (the peak) of lactation and declined from the peak average of 5.7 to 2.5 lb. in the 10th mo. The change in residual milk, with progressive lactation, may be due to a decreased active gland size, more complete excretion or a combination of these factors. The butterfat percentage in the residual milk was highest in early lactation and declined proportionally with quantity as lactation advanced. The highest monthly average was 16.4% and the lowest 11.6%. The fat content of residual milk from Jerseys and Holsteins bore the same relationship as the fat tests of their normal milks. The solids-not-fat percentage of the residual milk changed only slightly throughout the lactation. While the fat content of the milking following oxytocin-stripping was depressed 1.3%, the solids-not-fat content was not changed significantly.

**P33. Evaluation of mammary gland development of heifer calves.** J. H. BOOK, W. W. SWETT AND C. A. MATTHEWS, U.S.D.A.

The technique of examination and evaluation of mammary gland development of dairy calves

has now been applied to over 300 Holsteins and Jerseys in the Bureau of Dairy Industry Herd at Beltsville, Md., which have completed production records. For most of the ages studied (3, 4, 5 and 6 mo.), the grades assigned to the calves and their highest mature equivalent milk production records showed highly significant correlations. The grade assigned at 4 mo. of age gave the highest correlation for Jerseys and the grade at 6 mo. of age gave the highest correlation for Holsteins. There are some indications, however, that averaging the grades for 2 or more consecutive mo. increases the reliability of grades for mammary development as an indication of production. These results have been obtained from data on a single herd of relatively high and uniform production and maintained under controlled environmental conditions. Field tests are now under way in many areas to determine, under a variety of conditions, the reliability of evaluation grades for mammary development as a basis for selection.

**P34. Effects of udder innunction with diethylstilbestrol on mammary congestion in first-calf heifers.** JOSEPH MEITES, R. E. HORWOOD, E. P. REINEKE, C. S. BRYAN AND E. S. SMILEY, Michigan Agr. Expt. Station

In 1st-calf heifers, udder congestion is encountered frequently, with attendant discomfort to the animals and difficulties in management. It is believed that the congestion is caused primarily by a lymphatic and venous stasis and not by filling of the udder with milk. Inasmuch as estrogens have been shown to increase circulation in the mammary gland, it was considered probable that local application of an estrogen to the udder would prove beneficial in alleviating congestion.

On the 1st and 3rd days after calving, the udders of 8 heifers of the college herd were massaged with 200 mg. of diethylstilbestrol dissolved in 10 ml. of corn oil. An equal volume of blank corn oil was rubbed on the udders of 6 control 1st-calf heifers. Daily ratings of udder congestion were made in all heifers and chemical and microscopic examinations were made of the milk.

By the 3rd day postpartum, udder congestion in the diethylstilbestrol-treated heifers was reduced to half, whereas in the controls, udder congestion remained as marked as on the day of calving. Udder congestion continued to be reduced more rapidly in the hormone-treated than in the control heifers during the 2-wk. period of observation. The leucocyte count in the milk also was lowered more rapidly in the diethylstilbestrol-treated heifers. No effects of the hormone on milk production were noted.



**P35. Estimation of the thyroxine secretion rate without sacrifice of the animals.**<sup>1</sup> G. W. PIPES, C. R. BLINGOE AND KUANG-MEI HSIEH, Missouri Agr. Expt. Station

The present methods used for the determination of the thyroxine secretion rate require the sacrifice of the animals. For the larger domestic animals such as goats and cows, it is important to develop a method by which repeated measurements may be made during growth, pregnancy and lactation upon individual animals.

Considering the fact that conclusive evidence is available to demonstrate that thyroid secretion rate is governed by the thyroxine level of the blood, it seemed desirable to study the effect of graded thyroxine dosages upon thyroid function, in hopes of devising such a method. Maintaining the thyroxine level of the blood at or in excess of the normal secretion rate should result in minimum function of the thyroid.

In the present study, rats receiving dosages of thyroxine ranging from 0.5–10 µg./100 g. body weight were injected with 10–15 microcurries of I<sup>131</sup>.

In preliminary experiments, the rate of collection of radioiodine by the thyroid and the formation of protein bound radioiodine (thyroxine?) in the blood indicates that thyroid function reaches a minimum as the thyroxine dosage reaches the normal secretion rate.

<sup>1</sup> Contribution from the Dept. of Dairy Husbandry, Missouri Agricultural Expt. Station, Journal Series no. 1204.

**P36. Persistence of different causative organisms in mastitis infections.** LLOYD A. BURKEY, CECILIA R. BUCKNER AND W. W. SWETT, Bureau of Dairy Industry, U.S.D.A.

A study of bovine mastitis in the dairy herds maintained by the Bureau of Dairy Industry at Beltsville, Md., shows that infections by generally recognized mastitis infective organisms occur continuously in spite of symptomatic treatment. Furthermore, recurrent increases in numbers of infections occur irrespective of season or other evident factors. Although the incidences of infection by mildly infective streptococci and hemolytic staphylococci have been reduced during the last 2 yr., there has been an increase in the incidences of coliform and pseudomonad infections.

A study of both new and chronic infections shows that hemolytic staphylococci, *Streptococcus agalactiae*, pseudomonads, and *S. uberis* are often difficult to eliminate by a single treatment and infections by these organisms tend to persist and become established in the udder.

It is apparent from these results that the eradication of the major mastitis organisms in a large herd requires, in addition to therapeutic treatment, extreme vigilance in their detection and improved practices in herd management.

**P37. Factors involved in the Whiteside reaction.** W. E. PETERSEN, J. F. GRIMMELL AND I. A. SCHIPPER, Univ. of Minnesota

In a study of the reliability of various tests for mastitis, the Whiteside reaction was found to rate well. By centrifugation it was found that the factors responsible for the reaction resided in the precipitate and not in either the serum or fat. That the white cells *per se* were not responsible for the reaction was proven by negative reaction from concentrated cells from "negative" milk and isolated white cells from blood in healthy and infected cows with leucocytosis when added to normal milk. Exudates from abscesses added to normal milk gave positive reaction, as did expressed juice from either healthy or diseased mammary tissue.

Evidence that the reaction is caused by adsorption of fibrin on the white cells in the milk was obtained by the addition of blood plasma to negative milks reinforced with 1 million or more white cells/ml. Blood serum or plasma with anti-coagulants gave negative reaction when added to such fortified milks, presumably because the fibrin did not adsorb on the cells. Finely divided charcoal plus blood plasma added to cell-free milk serum gave positive reaction.

**P38. The influence of soybean hay on reproduction in the rabbit.** K. A. KENDALL AND G. W. SALISBURY, Univ. of Illinois

As a consequence of feeding rabbits soybean hay, a syndrome characterized by a reduced number of known pregnancies, small litters, high fetal mortality as shown by numerous partial resorptions, abortions and stillbirths, severe uterine bleeding, hemorrhage in other parts of the body, occasional fetal hemorrhage, prolonged gestation and death of does has been observed. A total of 71 matings resulted in 48 known pregnancies, of which 39 were carried to full term, producing 226 young or 5.8 young/litter.

When alfalfa, lespedeza and timothy were fed, 34 matings resulted in 32 known pregnancies, all of which resulted in litters carried to term, producing 267 young or an average of 8.3 young/litter.

**P39. A duodenal fistula for physiological studies in the bovine.** G. M. WARD, F. W. YOUNG AND C. F. HUFFMAN, Michigan State College

A fistula in the duodenum, approximately two-

thirds the distance from the pylorus to the entrance of the bile duct, was exteriorized immediately dorsal to the costo-chondral articulation of the 11th rib of a 700-lb. Holstein steer. The fistula was closed with a 2-piece plug shaped from Plexiglass. Collection of samples was effected by means of removing the fistula plug, allowing the lumen of that portion of the duodenum extending toward the bile duct opening to empty and then holding a vessel at the lower edge of the fistula to catch the chyme as it was propelled to the fistulous opening by peristaltic waves.

This technique, when combined with the use of a technique involving the use of a natural or artificial inert material as a "marker," offers a means of obtaining information regarding preintestinal digestion, absorption, excretion and synthesis.

**P40. Tests with sulphur dioxide for forage preservation.** J. B. SHEPHERD, H. G. WISEMAN, R. E. ELY, C. G. MELIN AND C. H. GORDON, Bureau of Dairy Industry; L. G. SCHOENLEBER AND L. E. CAMPBELL, Bureau of Plant Industry, Soils and Agriculture Engineering, Agricultural Research Administration; AND W. H. HOSTERMAN, Grain Branch, Production and Marketing Administration, U.S.D.A.

SO<sub>2</sub> was used in a number of forage preservation experiments at Beltsville in 1949. The field treating of freshly cut alfalfa in the swath with 70-80 lb. of SO<sub>2</sub>/acre did not increase the drying rate but did increase the rate of carotene loss during field curing. The stand or following growth of the crop was not affected. When injected into the top layer of well-tramped but uncovered silage, SO<sub>2</sub> delayed slightly but did not prevent formation of top spoilage.

SO<sub>2</sub> treatment of undercured baled alfalfa hay slowed down cell respiration for 1 or 2 wk., as judged by temperature measurements, and delayed heating, drying and molding. Experiments with hay in this "dormant" condition were conducted to improve the preservation of carotene and nutrients by barn curing on mow driers.

Baled alfalfa treated with SO<sub>2</sub> and cured on a mow drier without heat had a higher carotene content than similar untreated alfalfa; this effect was greater in uncured alfalfa than in field crushed alfalfa.

Two 3-ton lots of chopped alfalfa were cured on mow driers simultaneously without heat. One lot was treated with 25.8 lb. of SO<sub>2</sub>, admitted through the main air duct and blown through hay soon after storage. Although the hay was drier than usual when placed on the drier, the SO<sub>2</sub>-treated lot showed a preservation of over

5% more dry matter and 20% more carotene than the untreated hay.

**P41. A comparison of fecal nitrogen excretion rate, chromium oxide and "chromogen(s)" methods for evaluating forages and roughages.** P. G. WOOLFOLK, C. R. RICHARDS, R. W. KAUFMAN, C. M. MARTIN AND J. T. REID, Cornell Univ.

The use of fecal nitrogen as an index of digestibility and dry-matter intake was found to be inaccurate, due to the variability in the rate of nitrogen excretion which was not associated with the level of protein in the forages or roughages fed to calves and lambs. The average and range of fecal nitrogen excretion in grams/100 g. dry-matter intake for calves consuming the following feeds were: barn-cured mixed hay (9.72% protein), 0.72 (0.63-0.81); field-cured mixed hay (9.71% protein), 0.73 (0.71-0.78); mixed hay crop silage (10.85% protein), 0.88 (0.78-0.94); good field-cured mixed hay (11.13% protein), 0.73 (0.70-0.76) and timothy-mixed pasture grass: vegetative stage (16.15% protein), 0.71 (0.69-0.74); boot-to-early-head stage (12.00% protein), 0.66 (0.64-0.72) and full bloom stage (10.41% protein), 0.67 (0.64-0.70). The following values were found for lambs: oven-dried mixed hay (8.38% protein), 0.62 (0.58-0.64); barn-dried mixed hay (9.64% protein), 0.63 (0.59-0.65); field-cured mixed hay (9.41% protein), 0.67 (0.63-0.71) and mixed hay crop silage (11.08% protein), 0.91 (0.76-1.08). Chromium oxide also was found to be inaccurate as a reference substance for determining digestibility due to variation in recovery from the feces. Using a modification of Barnicoat's method of analysis, recovery in trials with calves averaged 99.63% (range 89.32-108.66%); in trials with sheep, 97.11% (range 86.72-109.09%). The level of "chromogen(s)" in individual samples of feces taken at various hours of the day was found to be comparable to that in the composite sample for a complete digestion trial. This indicates the possibility of using individual samples for determining digestibility and consumption following a suitable preliminary period.

**P42. A study of the use of chromium oxide and lignin as indicators of digestibility.** E. A. KANE, W. C. JACOBSON AND L. A. MOORE, Bureau of Dairy Industry, U.S.D.A.

In order to obtain data on the variation of the percentage of indicators in the feces of cows on ratio technique digestion trials the following experiment was undertaken.

Three cows which had received approximately 15 g. daily of chromium oxide for the previous

90 d. were used. During a 24-hr. period, each individual passage of feces from these cows was mixed and analyzed for dry matter, lignin and chromium oxide.

The ratios of the per cent of the indicator in the feed to the per cent of the indicator in the feces were calculated for each passage of feces from each cow for the 24-hr. period. The averages of the ratios were similar to those calculated for the preceding 9-d. total collection trial.

The range of the variation of these ratios for each of the 3 cows was 0.0516, 0.0866 and 0.0810, respectively, for chromium oxide and 0.0345, 0.0338 and 0.0290 for lignin. These variations are sufficiently large to cause some error in the dry matter digestibility coefficients. The graphs of the chromium oxide ratios of each of the 3 cows followed a similar pattern over the 24-hr. period. The lignin graphs of each cow, while in general agreement with each other, differed from those of the chromium oxide. The results of this investigation show that the time of sampling and the number of samples taken is important in obtaining accurate digestibility coefficients with the ratio technique.

**P43. The effect of dosage level and method of administration of DDT on the concentration of DDT in milk.** R. E. ELY AND L. A. MOORE, Bureau of Dairy Industry, AND R. H. CARTER, H. D. MANN AND F. W. POOS, Bureau of Entomology and Plant Quarantine, U.S.D.A.

Crystalline DDT was fed to several milking animals in dosages varying from 50-2000 mg. daily. The material was administered as (a) a soybean oil solution in capsules, (b) crystalline material in capsules and (c) a soybean oil solution mixed in grain. No significant differences in the DDT concentration in the milk occurred when the same dosage level was fed by these 3 methods of administration. Increasing the daily dosage of DDT resulted in comparable increases in the DDT concentration of the milk.

Two crops of hay made from alfalfa previously sprayed with DDT have been fed to 8 milking animals. The daily intake of DDT ranged from 109-727 mg. and progressive increases in the DDT concentration in the milk occurred with increasing intakes. The concentrations of DDT in the milk, however, were more than twice as high as when comparable dosages were fed as crystalline DDT.

When cows were fed DDT in oil solution containing the same proportions of 2 detergents as the material sprayed on alfalfa forage, the concentration of DDT in the milk was similar to that of control animals fed DDT in oil solution without the detergents. This indicates that the

detergent used on the treated forage is not responsible for increased absorption and excretion in the milk of DDT fed to dairy cows.

**P44. Studies on casein utilization by young calves by use of radioactive tracers.** G. P. LOFGREEN, Univ. of California

Casein has been tagged with radioactive phosphorus by the injection of lactating cows with inorganic phosphate solution containing P<sup>32</sup>. The tagged casein was incorporated into purified diets and fed to calves. Studies were made of blood levels of the isotope, its distribution in the body at 24 hr. after bleeding and its excretion in the feces.

The blood levels reached their maximum usually at between 15 and 18 hr. after feeding. There was little difference in the distribution of the isotope in the tissues when it was given in the organic form in casein, fed as inorganic phosphate, or injected directly into the blood stream as inorganic phosphate. Brain tissue consistently contained the lowest concentration of the isotope, while liver consistently was high. The rumen epithelium was higher in concentration than any other part of the digestive tract. By use of the ratio of nitrogen to radioactive phosphorus in the casein and in the feces it has been possible to calculate the metabolic nitrogen excretion of calves while being fed purified milk diets. Using the values thus obtained, the true digestibility of the casein was shown to be between 91 and 95%.

**P45. The biological activity of alpha and beta casein-thyroprotein.**<sup>1</sup> M. B. BEHRENS, G. W. PIPES AND C. W. TURNER, Missouri Agr. Expt. Station

Since the thyroidal activity of iodinated casein results from the iodination of tyrosine and the subsequent coupling of 2 molecules of diiodotyrosine to form thyroxine, it would seem likely that the iodination of  $\alpha$ -casein containing 8.1% tyrosine would produce a more active product than could be obtained from  $\beta$ -casein containing 3.2% tyrosine or from whole casein containing 6.3% tyrosine.

In the present investigations,  $\alpha$ -casein and casein fractions containing varying amounts of  $\alpha$ - and  $\beta$ -casein have been iodinated, and the resulting iodinated casein has been assayed biologically, chemically and by an isotope dilution method employing radioactive iodine.

<sup>1</sup> Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1207.

**P46. Thyroxine content of synthetic thyroprotein as determined by a radioactive isotope dilution**

**technic.** E. P. REINEKE, D. P. WALLACH AND L. F. WOLTERINK, Michigan State College

Previous studies have shown that the thyroidal activity of thyroproteins is related to their *n*-butanol-soluble iodine content. Although thyroxine can be isolated from such preparations in yields of 0.4–0.5%, no direct quantitative measurements have been available on their true thyroxine content. To obtain such a direct measure, a method was devised employing thyroxine labelled with radioactive iodine ( $I^{131}$ ).

The radioactive thyroxine was prepared by iodination of casein with  $I^{127}$  together with a small amount of  $I^{131}$ . Subsequent to hydrolysis with  $Ba(OH)_2$ , the radioactive thyroxine was recovered by crystallization procedures. A known amount of radioactive thyroxine was added to thyroprotein samples to be analyzed prior to their hydrolysis with  $Ba(OH)_2$ . Thyroxine was recovered and purified by repeated crystallization. The radioactivity of a weighed portion of this thyroxine then was determined, and the thyroxine content of the original sample was computed by calculating the extent of dilution of the radioactive thyroxine originally added by inert thyroxine recovered from the thyroprotein. Eleven thyroprotein samples analyzed contained from 1.13–3.25% apparent thyroxine by the *n*-butanol extraction procedure. By the isotope dilution technique, their true thyroxine content ranged from 0.293–0.753%. The average thyroxine value by the isotope dilution method was 22.9% that obtained by the *n*-butanol extraction method.

**P47. The influence of thyroprotein feeding on gains in body weight of dairy calves.** RALPH P. REECE, New Jersey Agr. Expt. Station

Thyroprotein was incorporated in dried milk solids supplemented with vitamins (Kaff-A) and fed at various levels to 4-d.-old calves for 10–88 d. Grain was fed *ad libitum* and all the hay that the calves would eat readily was fed twice daily. The feeding of 1 or 2 g. of thyroprotein daily for 88 d. had no significant influence on gains in weight in 6 pairs of calves. The average birth weight of thyroprotein-fed calves was 77 lb. and that of control calves was 80. At 3 mo. the average weight of the thyroprotein-fed calves was 178 lb. and that of the control calves was 173 lb. Three calves received 3 g. of thyroprotein daily for 10–14 d. This level of thyroprotein caused a loss in body weight and at 1 mo. of age the calves were below birth weight. Upon withdrawal of thyroprotein from the ration, the calves were fed whole milk and at 3 mo. their body weights were normal. A Jersey bull calf born in July failed to gain in weight when fed 2 g. of

thyroprotein daily for 17 d. The daily feeding of 3 g. of thyroprotein in conjunction with 50  $\mu$ g. of vitamin  $B_{12}$  resulted in a loss in body weight in a Jersey bull calf born in August. Thyroprotein withdrawal and continuation on Kaff-A resulted in marked increases in body weight. The per cent increase in body weight of a bull calf fed 2 g. of thyroprotein daily for 57 d. was 59, whereas that of a calf fed 2 g. of thyroprotein in conjunction with 50  $\mu$ g. of vitamin  $B_{12}$  was 93. The gain in weight of a heifer calf fed 50  $\mu$ g. of vitamin  $B_{12}$  in conjunction with Kaff-A was no greater than that of a calf fed Kaff-A with no supplementation.

**P48. The use of x-rays for the detection of rickets in calves.** J. W. THOMAS, Bureau of Dairy Industry, U.S.D.A.

Accurate diagnosis of rickets in calves has not been possible because the physical and/or chemical methods employed have not been sufficiently sensitive and applicable. This has been especially true under field conditions where obvious symptoms of rickets rarely occur.

By the use of x-rays, following the procedure outlined by Bechdel *et al.*, it was found that the width of the epiphyseal cartilage of the ulna of normal calves follows a definite pattern. From birth to 8 mo. of age it gradually ossified and closed. The roentgenogram was taken of the left ulna at about a 75° angle from front view. The results of this technique were correlated with studies made on plasma Ca, P and phosphatase and with bone ash of over 75 calves. This technique offered an excellent means of detecting rickets and it can be used advantageously where it is difficult to obtain data by the usual methods.

This technique indicated that calves allowed limited access to sunshine and fed barn-dried alfalfa or alfalfa silage as their sole roughage were free from rickets.

Another group of calves was kept in a well-lighted barn and fed skim or whole milk, shark liver oil and grain. In mid-December, when they were 68–118 d. of age, they showed evidence of rickets as shown by roentgenograms. Only 1 calf showed gross symptoms of rickets. Rapid closure was effected by feeding viosterol; slow closure by limited winter sunshine.

**P49. An approach to the problem of the etiology of ketosis in dairy cows.** B. C. HATZIOLOS AND J. C. SHAW, Univ. of Maryland

Some of the more important histopathological findings observed in a significant per cent of cases have been cystic degenerations, atrophy and fatty degeneration of the anterior lobe of the pituitary and enlarged adrenals with degenerative areas

in the outer zones. An adrenal with polymorphonuclear infiltration in the zona fasciculata and another with an abnormal growth in the cortex have been observed. The response of ketotic cows to adrenal cortical extracts and one case of response to cortisone also indicates that the pituitary-adrenal system is involved. Numerous abnormalities have been observed, such as inflammation or congestion of the abomasum and small intestine, ulcer, metritis and lipofibroma around the kidney. It is considered significant that in practically all of the cases the cows exhibit typical symptoms of ketosis and respond to intravenous injections of glucose.

A tentative proposal is made that ketosis in cows is basically a disease of the so-called adaptation syndrome, brought on by various stresses during a period when the organism is overworked.

**P50. Kentucky 31 fescue as a dairy pasture in northern Ohio.** AVERY D. PRATT AND JAMES L. HAYNES, Ohio Agr. Expt. Station

Kentucky 31 fescue was seeded with alfalfa and ladino clover in 1948 and first grazed in 1949. Good stands of ladino and Kentucky 31 resulted with only a small admixture of alfalfa. Jersey heifers made from 0.2–0.4 lb. gain in body weight daily while grazing it, as compared to 1.7 lb. when grazing bluegrass and ladino or meadow crop mixtures. Milking cows in reversal experiments ate more hay, produced less milk and lost more weight when grazing Kentucky 31 and ladino than when grazing bluegrass and ladino. Again in comparison with meadow fescue, Birdsfoot trefoil and ladino, the Kentucky 31 and ladino gave similar results. A reversal experiment with 1 group of cows on Kentucky 31 and ladino and the other in dry lot resulted in slightly higher milk production when in dry lot. Silage consumption was nearly the same for both lots. Those in dry lot ate more hay and gained more in body weight.

Ohio farmers are advised not to seed Kentucky 31 fescue until more favorable results are obtained.

**P51. Fourteen years with supplementary pastures.** N. R. THOMPSON AND C. W. HOLDWAY, Virginia Agr. Expt. Station

Various legumes and grasses were grown singly and in combinations over a period of 14 yr. and grazed by dairy cattle. Plots of bluegrass were grazed the 1st 5 yr. Estimated total digestible nutrient yields/acre/season included 2,186 lb. for alfalfa-bromegrass, 2,019 for alfalfa-orchard grass, 1,946 for ladino clover, 1,678 for Korean lespedeza-orchard grass, 1,569 for bluegrass, 1,424 for crimson clover-Abruzzi rye-

Italian rye grass, 1,368 for Korean lespedeza-orchard grass-sweet clover and 986 for Korean lespedeza-bromegrass. Average production in pounds of 4% fat-corrected milk/cow/day on the above pastures was 33.1, 27.1, 30.8, 29.3, 21.7, 22.3, 0, and 28.7, respectively. Values for milk production are not strictly comparable, since they were made by cows in various stages of lactation and over a number of years, but may be assumed to indicate some possibilities of the various pastures for milk production.

Some pastures were available for grazing throughout the season, others for only short periods. Alfalfa-orchard grass and alfalfa-bromegrass were available continuously from late April to late Sept. Crimson clover-Italian rye grass and crimson clover-Abruzzi rye were available principally in April and May and occasionally in Oct. and Nov. Sudan grass was available for grazing in July and Aug. Several pastures showed peaks in nutrient production in May and June.

**P52. The effects of partial replacement of alfalfa hay with concentrates.** K. A. KENDALL AND R. D. ENGBERSON, Univ. of Illinois

Four groups of 2 cows each were fed alfalfa hay and salt for a period of 8 wk. In the 2-wk. experimental period that followed, each cow within a pair was fed a reduced amount of alfalfa and 1 other nutrient source. The other nutrient source was either 7 lb. ground corn, 7 lb. starch, 7 oz. corn oil or 7 oz. coconut oil replacing in the daily feed allowance 11, 12.5, 2.5, or 2.5 lb. of alfalfa hay, respectively. T.D.N. intakes for the experimental period were from 4–8% less than those of the preceding 2-wk. basal period of alfalfa hay feeding.

The per cent increase (+) or decrease (–) in pounds of 4% F.C.M. produced per pound of T.D.N. consumed in the experimental period, as compared with that in the basal period, was for corn, +6.0 and –47.0; starch, –5.2 and –14.9; corn oil, 0 and +5.0; and coconut oil, +8.8 and +9.0.

**P53. Efficiency of silage and extra grain feeding for maintaining summer milk production.** DWIGHT M. SEATH AND RALPH F. ELLIOT, Kentucky Agr. Expt. Station

Feeding either corn silage or an extra amount of grain mixture to Holstein and Jersey milk cows on pasture from Aug. 1 to Sept. 11, 1949, aided in preventing the late summer decline in milk production. The benefits probably were not large enough to be economically profitable, although some of the benefits that might have resulted were nullified by the presence of better-than-average grazing.

In terms of 4% FCM, the daily milk production per cow for the 42-d. experimental period, when compared to that for the 14-d. preliminary period, declined 2.73 lb. for cows receiving the check ration, declined 1.25 lb. for those receiving the silage as supplement, and increased 1.12 lb. for cows receiving the extra allowance of the grain mixture.

Cows fed the check ration received a 16% grain mixture at the rate of 0.6 lb. for each 1 lb. milk in excess of 10 lb. daily for Jerseys and 0.4 lb. for each 1 lb. milk in excess of 15 lb. for Holsteins. Those receiving silage were fed grain, as were the check group, but in addition were allowed all the silage they would clean up. Their average daily consumption was 15.4 lb./cow and this contributed 18% of the calculated total digestible nutrients (TDN) required by the cows.

Cows receiving extra amounts of the grain mixture as a supplement consumed 2.7 lb. of grain/cow daily in addition to the amounts required when calculated as for the check group. In terms of TDN, this was 17.3% of their calculated requirements.

Body weight data for the cows during the experimental period showed that those in the check group gained 36 lb./cow, those receiving silage gained 70 lb., and those receiving extra grain gained an average of 82 lb./cow.

**P54. Rates of grain feeding for dairy heifers on temporary and permanent winter pasture.** S. H. MORRISON AND J. F. DEAL, Univ. of Georgia

Twelve purebred Jersey heifers ranging in age from 8-12 mo. of age were divided into 3 outcome groups of 4 animals each. All groups received pasture and U. S. no. 1 lespedeza hay, free choice. Two groups were on temporary pastures comprised of a mixture including Italian rye grass, oats and crimson clover. The 3rd group was on a permanent winter pasture comprised of a mixture of ladino clover and fescue (Alta and Ky. 31).

One control heifer in each group was fed 2 lb. of grain daily (12% total protein) throughout the entire experiment. Each of the other 3 animals in each group received 1 of the following amounts of grain in a Latin square design during each experimental period of 5 wk.: 0, 2, or 4 lb. There was a transition period of 1 wk. between the experimental periods.

Data will be presented on the actual hay consumption, grain consumption, weights and height at withers of the heifers on the 2 types of pasture. Tentative recommendations will be made for grain feeding rates on both types of pasture.

**P55. Kudzu and fescue-ladino clover silages for**

**dairy cows.** W. A. KING AND J. P. LAMASTER, So. Carolina Agr. Expt. Station

Kudzu and fescue-ladino clover silages were compared to corn silage in a 16-wk. continuous feeding trial. The kudzu was ensiled in Sept., 1949, with 61 lb. of molasses added/ton of green material. The fescue-ladino clover (70-30%) silage was ensiled in May with 65 lb. of molasses added/ton. Eight cows in each of 3 groups were fed the respective silages, Kobe lespedeza hay and a concentrate mixture. The daily silage consumption per cow averaged 45.6 lb. corn silage, 44.4 lb. kudzu silage and 47.8 lb. fescue-ladino clover silage for the respective groups. The fescue-ladino clover silage was as palatable as the corn silage, whereas the kudzu was less palatable.

The average daily milk production (4% F.C.M.) per cow was 34.9 lb. for the corn silage group, 35.1 lb. for the kudzu silage group and 34.6 lb. for the fescue-ladino clover silage group. The gains in live weight per cow averaged 48, 8 and 38 lb., respectively.

The carotene content of the corn silage averaged 62 p.p.m. of dry matter; the kudzu silage, 166 p.p.m.; and the fescue-ladino clover silage, 240 p.p.m.

**P56. Farm grains vs. a medium protein concentrate mixture for cows.** K. F. GARDNER, Univ. of Illinois

Thirteen cows of the 5 major dairy breeds received, over an 18-wk. period, a 9.5% total protein concentrate composed of 60% corn-and-cob meal, 37.5% ground oats, salt and additional bone meal. A similar control group received added soybean oilmeal and linseed oilmeal to provide a 14% protein mixture. Medium alfalfa hay and corn silage were fed at rates of 1 and 3 lb., respectively, /100 lb. body weight daily. Concentrates were fed at the middle rate recommended in the grain feeding table shown by Morrison. The cows were classed as medium producers, since none exceeded 45 lb. FCM daily.

The low-protein cows averaged 27.11 lb. FCM daily and the medium protein group, 26.45 lb. Analysis of variance showed that this difference was not statistically significant.

**P57. The relative palatability of expeller and extracted linseed meal in dairy cow rations.** N. N. ALLEN, Univ. of Wisconsin

Using 16 cows, a palatability test was run comparing a 34% protein, 4.0% fat expeller meal with a 37% protein and 0.5% fat extracted meal. The test rations contained 300 parts each of oats, corn, and linseed meal and 150 parts of wheat bran. During a preliminary period, 8 cows were fed the feed containing expeller meal,

while the others got the feed containing extracted meal. During the 10-d. test period, using a divided feed box, they received  $\frac{1}{2}$  of their usual allotment of concentrate in each form, having free choice. They were observed closely for any evidence of preference. Of the 160 feedings to the cows conditioned to the expeller meal, in 14 there was some evidence of preference for the expeller and in 8 for the extracted meal. Of those 160 feedings to cows conditioned to extracted meal, in 2 cases there was evidence of preference for expeller and in 5 for extracted meal. Of the total 320 feedings, in 16 there was evidence of preference for expeller and in 13 for extracted meal. In no case was there any pronounced objection to either feed and, in most cases, the evidence of preference was slight. The conclusions were that there is no practical difference in palatability of the expeller and extracted meals when used in a dairy cow concentrate at the level of 28%.

**P58. Preliminary observations on the effects of nutrition on the quality and quantity of bovine semen.** II. H. OLSON, W. E. PETERSEN, T. W. GULLICKSON AND J. N. CUMMINGS, Univ. of Minnesota

One set of identical triplet bulls, *T*, *D* and *H* were placed on 3 levels of nutrition. *T* was fed 30% below normal, *D* was fed normally and *H* fed 30% above normal. *D* was used as the guide for *T* and *H*. Two ejaculates were collected from each bull at weekly intervals during an 8-mo. period. These samples were tested for quality and quantity by the following tests: volume, density, total number of sperm per ejaculate, motility, methylene blue reduction and abnormal sperm.

*T* produced the smallest volume of semen, whereas *H* produced the largest volume. The second ejaculates were larger than the first. Density tests indicated no real differences between the bulls. First ejaculates tended to have a greater density than the second. *T* produced the lowest number of sperm per ejaculate, but little difference was found between *D* and *H*. More sperm were found in the second ejaculates than the first. *T* was found to have the poorest motility and little difference was noted between *D* and *H*. First ejaculates were found to have the best motility ratings. The methylene blue reduction test indicated *T* as producing the poorest semen and *D* the best semen; *H* was found to be just below the reduction time of *D*. The first ejaculates were found better than the second. There was no marked difference between the bulls on number of abnormal sperm produced. However, *T* produced the most abnormal sperm

and *H* the least. The second ejaculates contained more abnormals than the first. *H* exhibited the most libido and *T* the least.

The results under the conditions of this experiment indicate that a bull fed a below normal ration (*T*) produces semen of poorer quality and smaller quantity than bulls fed normal (*D*) or above normal (*H*) rations.

**P59. A preliminary report on the effect of the site of semen deposition on fertility in artificial insemination.** G. W. SALISBURY AND N. L. VANDEMARK, Univ. of Illinois

The results of 2 limited though carefully controlled experiments involving 936 cows suggested that cervical deposition of semen was as satisfactory as deposition either into the body or the horns of the uterus. The inseminating catheter was guided into the female reproductive tract by manipulation of the tract through the rectal wall. The conception rate was much higher for insemination into the cervix than had been reported for other experiments in which cervical insemination was accomplished by aid of a speculum.

To obtain more data on the appropriate site of semen deposition, an experiment was conducted involving 18 technicians of the Northern Illinois Breeding Association, 3 time periods of 1 mo. each, and insemination into the cervix, into the body of the uterus, or into each of the uterine horns. Six 3 x 3 Latin squares made up the design and several thousand cows were inseminated. Preliminary and as yet inconclusive results at the time of writing indicate that there is no appreciable advantage to be gained by insemination beyond the cervix.

**P60. Spermatozoan transport in the reproductive tract of the cow.** N. L. VANDEMARK AND A. N. MOELLER, Univ. of Illinois

Investigations are being carried out to determine the minimum time required for spermatozoa to reach the ovarian portion of the oviduct after deposition in the cervix of the cow by artificial insemination. Ten cows were slaughtered at various intervals after insemination, and the uterus and oviducts were clamped off at various locations. While carefully controlling the temperature of the tract, fluid samples were withdrawn from each isolated location by means of a syringe and needle and examined microscopically for the presence of spermatozoa. The interval from insemination to clamping off after slaughter has been gradually diminished from 140 min. down to 30. Spermatozoa of varying degrees of activity have been used and were found in the ovarian portion of the oviducts at the shortest interval studied. To eliminate the possibility

that spermatozoan transport in the first of this study was a result of the slaughtering procedure, the right horn and oviduct of each of a second series of cows were clamped off before slaughter through a surgical opening in the flank. Using this technique, spermatozoa have been found in the ovarian portion of the oviduct when the interval between insemination and clamping off was 11 min.

**P61. The effect of sterile copulation on the time of ovulation in dairy heifers.** GERMAIN G. MARION, VEARL R. SMITH, THOMAS E. WILEY AND GEORGE R. BARRETT, Univ. of Wisconsin

The effect of sterile copulation on the time of ovulation in 25 dairy heifers was determined. The time of ovulation was established from the time when the heifer was observed to go out of estrous. Each heifer was observed through 4 clinically normal estrous periods which were alternately designated as control and experimental periods; thus, each animal served as her own control. During each of the 2 experimental periods, the heifer was serviced once by a vasectomized bull.

The average time interval between the end of heat and time of ovulation following non-service or control periods was 9.91 hr., while this interval for experimental periods was 7.73 hr. Statistical analysis showed this difference of 2.18 hr. to be highly significant.

**P62. Measuring reproductive efficiency in dairy cattle.**<sup>1</sup> F. A. BUSCHNER, R. E. JOHNSON, C. I. BLISS AND A. A. SPIELMAN, Storrs Agr. Expt. Station, Univ. of Connecticut.

The breeding records from the University of Connecticut dairy herd were analyzed statistically in order to devise a measure of reproductive efficiency which takes into account the various measurable factors affecting the reproductive life of a cow. The effect of 4 factors upon the age at 3rd calving was studied, namely, age in days at first breeding, the interval from first breeding to conception for the first reproductive cycle, interval from calving to first breeding for the second and third reproductive cycles and interval from first breeding to conception for the second and third reproductive cycles. Partial regression coefficients for each factor were calculated for each breed separately and used to compute an estimated age at 3rd calving. More than 99% of the variability in age at 3rd calving was accounted for by these partial regression equations, although in the aggregate they represented only  $\frac{1}{2}$  of the total period. A new score for reproductive efficiency then was based upon the age in days at 3rd calving. Cows reaching this age before the herd average are the more efficient reproducers, if the

effect of abortions is excluded. The new measure, in units of the standard deviation from the herd mean, shows an animal's relative position in the herd and facilitates the comparison of animals from different herds. This measure can be estimated with practicable reliability as soon as a heifer is safely in calf from partial regression equations based upon age at first breeding and the interval from first breeding to conception.

<sup>1</sup>Supported in part by funds provided under Section 9B3 and 10A, Research and Marketing Act, 1946, in cooperation with the Bureau of Dairy Industry.

**P63. The sex ratio in calves resulting from artificial insemination.** K. E. GARDNER,<sup>1</sup> Univ. of Illinois

This study was prompted in part by a recent paper reporting that the sex ratio in rats was affected by the length of the interval between ovulation and insemination. In the artificial insemination of dairy cattle the cow probably is bred later in the heat period than would be the case in natural breeding. This should result in a shorter-than-normal time interval between insemination and ovulation, since the cow normally ovulates postestrus.

A sex ratio at birth of 100 females to 105.89 males (917 males to 866 females) was found in a survey covering 1,783 single-born Holstein calves resulting from artificial insemination. The data were obtained in studies conducted in 26 herds which kept accurate and complete breeding and calving records. This sex ratio is very similar to the ratio of 100 females to 106.2 males obtained by Johansson in his studies of 125,000 calf births from natural service in Scandinavian herds. A summary of the twins born in these Illinois herds showed that 9 calvings produced male twins, 21 calvings produced female twins, and 27 calvings produced mixed twins, making a total of 1897 calves in the study.

<sup>1</sup>The assistance of L. S. Zuckerman is gratefully acknowledged.

**P64. Artificial breeding in Alaska and the effect of extra light during the short winter days.** WILLIAM J. SWEETMAN, Alaska Expt. Station, Palmer

In Matanuska Valley the possible sunlight on Dec. 21 is 5 hr. and 28 min. In the winter of 1948-49 a number of farmers gave their cows 14 hr. of light/day. The others received only the natural light plus what was needed to do chores. This amounted to an average of 8 hr. or less/day. Of 111 cows receiving additional light bred from Oct. 1, 1948, to May 31, 1949, the conception rate was 53.6% and the conception



rate of 93 cows in the natural light group was 48.6%. Services per conception were 1.86 in the additional light group and 2.05 in the natural light group. This is a significant difference.

The seasonal percentages of non-returns was as follows: fall of 1948, 55.2%; winter of 1948, 44.0%; spring of 1949, 55.0%; summer of 1949, 48.3%; and fall of 1949, 54.6%.

Of 1178 inseminations from Aug., 1948, to Jan., 1950, there were 584 1st services, 320 2nd services, 157 3rd services, 80 4th services and 34 5th services. There were 3 services beyond the 5th. The corresponding percentages of non-returns were 50.7, 57.2, 56.1, 50.0 and 29.4. The over-all percentage of non-returns was 52.4.

**P65. Nutritive value of crops and cows' milk as affected by soil fertility. II. The amino acid composition of colostrum and milk.<sup>1</sup>** C. W. DUNCAN, K. M. DUNN and GERTRUDE I. WATSON, Michigan Agr. Expt. Station

An experiment was initiated in 1945 to study the characteristics of certain plant species grown on natural soils seriously depleted of mineral nutrients and on the same kind of soil with the addition of adequate amounts of lime and mineral fertilizers. The feeds grown on the fertilized and depleted soils are being fed to 2 groups of cows as their sole ration. The composition of the crops, milk and blood has been determined at regular intervals since the beginning of the experiment.

The amino acid content of the colostrum, 60-d. composited milk and terminal milk samples of the 2 groups of cows has been determined by microbiological methods for arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. These data have been collected from the 1st through the 4th lactation period. No difference has been obtained in the amino acid pattern of either group for the 0-hr. or 24-hr. colostrum samples or between the Jersey or Holstein breeds, with the exception of arginine, which tends to be higher in the colostrum of the cows receiving the unfertilized feed. The threonine content of the 60-d. composited milk and terminal milk samples was markedly lower than the colostrum in both the Jersey and Holstein breeds, but this phenomenon can not be attributed to the feeds.

With the exceptions noted, there is no essential difference in the amino acid composition of the colostrum and milk of the 2 groups of cows that can be attributed to feeds grown on depleted soil or on highly fertilized soil of the same type.

<sup>1</sup> This work was supported in part by a grant from the National Dairy Council on behalf of the American Dairy Association.

**P66. Dairy cow stall studies.** I. D. PORTERFIELD, GEORGE HYATT, JR., D. P. BROWN AND A. D. LONGHOUSE, West Virginia Univ.

A comparison of Holstein cows kept in 2 types of stalls was made at West Virginia University. There were 7 "tie chain" stalls each 66 in. long and 42 in. wide and 7 "comfort" stalls 84 by 49 in. with an adjustable "cross bar" in each one. Data were collected between Oct. and May in 1947, 1948, 1949 and 1950.

Eight cows in "comfort" stalls produced 2-12 lb. more milk (2X M.E. basis) daily for a minimum of 14 wk. than when they were in "tie chain" stalls. In 2 trials, 5 of 7 cows remained cleaner in the "comfort" stalls than they did in "tie chain" stalls. Difference in daily requirements for bedding for each type of stall was not statistically significant. Nine observations showed no significant difference in time needed to clean the 2 types of stalls. No cases of mastitis have been encountered.

Observations for leg injuries were made on the 14 cows 3 times each week. Two mo. after cows were stabled 7 in the "tie chain" stalls showed injuries as compared with 1 in "comfort" stalls.

Cows in the "tie chain" stalls spent 8.8 hr./24-hr. period lying down as compared to 10.2 hr. for cows in the "comfort" stalls. The difference was statistically significant.

**P67. Calf losses from disease.** H. P. DAVIS, Univ. of Nebraska

The dairy herd at the University of Nebraska has had an unusually continuous system of management, with only 3 persons responsible for the management practices in 44 yr. In a study of the births and final disposal of calves from 4 dairy breeds, Holstein, Jersey, Ayrshire and Guernsey, the abortions, live births, sex ratio, twins and losses from disease were tabulated and reduced to percentages. Altogether, 2,362 calvings were involved with a total of 2,428 calves being born, with a sex ratio of 51.48% males to 48.52% females. There were 149 abortions or 6.31% of the calvings. Sixty-six twins were born or 2.79% of calvings. Altogether, 2,166 calves were born alive or 89.21% of calvings. Disease losses were divided into digestive, respiratory, infectious and other causes.

Thus, there was a total of 14.77% loss from disease during the first 2 yr. of life. From 1 to 3 mo. the losses due to these causes were 1.80, 3.55, 1.66 and 5.12%, respectively, from 4 to 6 mo. they were 0.37, 0.88, 0.23 and 0.23% respectively, and from 7 to 23 mo. they were 0.18, 0.23, 0.23 and 0.28%, respectively.

**P68. Relation of production records on cows and efficient management of the dairy farm.** LEO R. FRYMAN, Univ. of Illinois

Using 3-yr. records from 65 dairy farms in northeastern Illinois, 5 factors were found to have a significant influence on both the rate earned on the total investment in the farm business and net earnings per acre. These 5 factors are: returns for each \$100 spent for feed for dairy cattle, level of production of the cows in the herd, size of the herd, labor costs per cow and crop returns on tillable land. Farms which had tested in a DHIA for 10 or more yr. were compared with similar farms which had not tested in DHIA. The 2 groups of farms were similar as to crop returns on tillable land, labor costs per cow and size of the herd.

Herds tested in DHIA had a higher milk and butterfat production, higher returns above feed costs and higher returns for each \$100 spent for feed for dairy cattle than non-DHIA herds. They also had a higher rate earned on investment, higher net earnings per acre and higher operator's earnings.

**P69. Relation of gestation to body weights of cows on long-time feeding trials.** R. B. BECKER, P. T. DIX ARNOLD AND SIDNEY P. MARSHALL, Florida Agr. Expt. Station

Eight non-gravid uteruses (severed at the os uteris) of Jersey cows averaged 1.4 lb. Thirty-five Jersey, 1 Guernsey and 1 Jersey-Guernsey fetuses from 32 to 279 d. in gestation, with accompanying placentae, fluids and empty uterus weights, may serve as a basis for computing corrections in weights of Jersey cows on long-time feeding trials. Changes in weight of uterus and contents were negligible up to past 60 d. in gestation. From 90 d. onward, they amounted to 5, 12, 22, 31, 43, 75 and 110 lb. at progressive 30-d. intervals, including the 270th day. At full term, the weight increase attributable to gestation amounted to 122.2 lb., assuming a 55 lb. calf, 15.8 lb. placenta, 38.2 lb. of fluids and allowing 14.6 lb. for involution of the uterus after calving.

Weight corrections would minimize error that arises when gross weight changes of cows advancing in gestation are computed at 3.53 and 2.73 lb. of total digestible nutrients/lb. of body gain or loss, respectively. Such corrections also would reduce a source of serious error arising from attempts to maintain "constant body weight" by adjusting concentrate offerings to pregnant cows on long-time feeding trials.

**P70. The influence of oestrus on weights of Holstein and Jersey heifers.** H. B. MORRISON, Kentucky Agr. Expt. Station

Heifers weighed 3 consecutive days on a pasture experiment usually weighed less on the day they were in heat. Records revealed 241 instances in which a heifer was weighed when in heat. Of these, 80 were in heat on the 3rd, 91 on the 2nd, and 70 on the 1st d. weighed. Of 171 instances where weights were available the day before and the day in heat, the average decrease in weight was 19.5 lb. The weight differences varied from a drop of 66 lb. to an increase of 20 lb., and standard deviation was  $\pm 13.83$  lb. Of 171 instances, 160 (93.6%) weighed less on the day in heat, 1 (0.6%) weighed the same and 10 (5.8%) weighed more. Of 161 instances where weights were available for the day in heat and the day after, the average increase in weight the day after heat was 16.8 lb. The differences in weight ranged from a decrease of 26 lb. to an increase of 70 lb. and showed a standard deviation of  $\pm 16.96$  lb. An increase in weight the day following heat occurred in 135 cases (83.8%), in 4 cases (2.5%) the heifers weighed the same and in 22 cases (13.7%) the heifers weighed less on the day following than on the day in heat. The average weight of the heifers on the day in heat was 625 lb.

**P71. The effect of a combination of penicillin, streptomycin and sulfanilamide upon the fertility of bull semen.** J. O. ALMQUIST AND P. W. PRINCE, Pennsylvania State College

A field trial was conducted at the First Pennsylvania Artificial Breeding Cooperative, utilizing semen from all bulls in active service to determine the effect of penicillin, streptomycin and sulfanilamide upon fertility. Three egg yolk-citrate diluters containing: (a) 1,000  $\mu$ g. streptomycin sulphate/ml., (b) 1,000 units penicillin G plus 1,000  $\mu$ g. streptomycin/ml. and (c) 1,000 units penicillin G plus 1,000  $\mu$ g. streptomycin plus 3 mg. sulfanilamide/ml. were compared with yolk-citrate diluter containing 3 mg. sulfanilamide/ml. (control). Each semen sample was diluted with yolk-citrate and divided into 2 equal portions. One-half received 3 mg./ml. sulfanilamide, while the other half received 1 of the above 3 treatments. The results were based on 6-mo. non-returns involving 296 ejaculates used on a total of 11,715 first and second service cows. The over-all results showed significant mean increases of 5 percentage units for diluters a and c over the control diluter, while diluter b showed an insignificant increase of 2 percentage units. Further analysis, however, revealed that the effectiveness of these diluters was dependent upon the relative fertility levels of the bulls. When divided into high and low fertility groups (based on the average non-return rate for the control

diluter), diluter *b* now produced a significant increase of 7 percentage units among the low fertility bulls, while diluters *a* and *c* brought about highly significant increases of 11 percentage units. On the other hand, in the high fertility group no significant difference was found between the control diluter and diluters *a*, *b* or *c*.

**P72. Bull semen toxicity of various salts, brands, and lots of penicillin, streptomycin, aureomycin and chloromycetin.** JAMES G. SYKES AND JOHN P. MIXNER, New Jersey Agr. Expt. Station

Two salts including 4 brands (2 lots each) of penicillin, 3 salts including 3 brands (2 lots each) of streptomycin, 2 salts of aureomycin and chloromycetin were tested for their semen toxicity. Six dosage levels of each antibiotic were added to diluted semen which was stored at 5° C. for a 15-d. period. The criterion of toxicity used was the estimated per cent of motile spermatozoa on days 5, 10 and 15.

No differences in toxicity could be demonstrated between the sodium and potassium salts of penicillin or among any of the 4 brands or 8 lots of penicillin studied. Streptomycin in the form of the sulfate, hydrochloride and calcium chloride complex represented the 3 brands and 6 lots studied. Again, significant toxicity differences could not be demonstrated among any of these salts (or brands) and lots of streptomycin. No difference was found between the relative toxicity of aureomycin base and the hydrochloride. However, aureomycin (base or hydrochloride) is relatively toxic to spermatozoa as the initial toxic level is in the general range of 125 to 250 µg./ml. of diluted semen. Chloromycetin would seem to have a relatively low level of toxicity, as the initial toxic levels seemed to be between one and 2 mg./ml. of diluted semen.

**P73. The influence of aureomycin upon the livability and bacterial content of bull semen.** R. M. MYERS, J. O. ALMQUIST AND P. W. PRINCE, Pennsylvania State College

Six levels of aureomycin hydrochloride (Lederle) ranging from 50–1,000 µg./ml. of diluted semen were studied, using 19 semen samples. All levels caused significant toxic effects on motility when compared with portions of the same semen samples containing no aureomycin or 1,000 units each/ml. of penicillin G (Pfizer) and streptomycin sulphate (Pfizer). Bacterial plate counts of 18 semen samples were made after 0, 4, 8 and 16 d. of storage at 4.5° C. Concentrations of aureomycin from 100–1,000 µg./ml. brought about effective control of bacterial growth. However, the 50 µg. level was not as efficient as the higher concentrations. The antibacterial ac-

tivity of 1,000 µg. of aureomycin/ml. compared favorably with the combination of 1,000 units each/ml. of penicillin and streptomycin. Assays showed that aureomycin was relatively stable in diluted semen stored up to 16 d. at 4.5° C. Diluted semen samples assayed after 8 and 16 d. of storage at room temperature showed a marked decrease in aureomycin activity.

**P74. Hyaluronidase and fertility of dairy bull semen.** JAMES E. JOHNSTON AND JOHN P. MIXNER, New Jersey Agr. Expt. Station

It is believed that hyaluronidase is an enzyme which acts in the physiological manner to allow spermatozoa to make contact with the ovum. The purpose of this study was to determine whether a significant relationship existed between the hyaluronidase content and fertility of dairy bull semen diluted at a rate of less than 1: 100.

Hyaluronidase assays on semen were made within 1 hr. of ejaculation and after incubation for 24 hr. at 37° C. under toluene. Fertility estimates were based on 60- to 90-d. non-returns to 1st and 2nd service breedings and include the following 3 categories: (a) 82 semen samples from 21 bulls to which 10–19 breedings were made/sample; (b) 105 semen samples from 18 bulls to which 20 or more breedings were made/sample; and (c) a total of 187 semen samples from 24 bulls to which 10 or more breedings were made/sample for a total of 4,657 breedings with average % N.R. of 66.1.

Data on initial and 24-hr. hyaluronidase levels were correlated with data on fertility in each group on a "total," "between bull" and "within bull" basis. No correlations secured had statistical significance and it may be concluded that no significant relationship exists between the hyaluronidase and fertility levels of semen from bulls of relatively high fertility when the semen is diluted at a rate of 1: 100 or less.

**P75. A comparison of two streptomycin compounds used in diluted bull semen.** H. L. EASTERBROOKS, P. HELLER, W. N. PLASTRIDGE, E. L. JUNGHER AND F. I. ELLIOTT, Storrs (Conn.) Agr. Expt. Station

The purpose of this study was to test dihydro (DH) streptomycin sulfate and streptomycin calcium chloride complex (CCC) for evidence of incompatibility with semen diluter buffers and for their comparative value in producing increased fertility rates. Graduated concentrations of the 2 streptomycins were added to various concentrations of citrate and phosphate buffers. Visible precipitation occurred in all tubes containing phosphate buffers to which streptomycin CCC in excess of 100 units (µg.)/ml. had been added.

Ten ejaculates used by the Connecticut Artificial Breeding Association over a period of 5 consecutive weekends in Oct. and Nov., 1949, were subjected to a split sample study involving the 2 drugs at a level of 500 units/ml. of diluted semen. Comparisons were based on 60- to 90-day non-return (N.R.) to 1st service percentages. Semen treated with DH streptomycin was used to inseminate 394 cows with a % N.R. of 72.34, as compared to 68.03 for semen containing streptomycin CCC used on 398 cows. Although this 4.25% N.R. increase for the DH streptomycin treated semen was not statistically significant ( $P < 0.20$ ), it appears that it may be the drug of choice when streptomycin is added to semen diluters.

**P76. Results of breeding dairy cows with egg yolk citrate and Ortho semen diluters.** VICTOR HURST, South Carolina Agr. Expt. Station

The Clemson Agricultural College bull stud

supplies diluted semen for approximately 400 breeding females in the College herd and also for 15,000 cows in 12 cooperating artificial breeding units in South Carolina. During the past 1.5 yr. these cows have been bred with semen extended in a diluter composed of 40% egg yolk and 60% 0.1 M sodium citrate. Although results have been variable, monthly average conception rates of 69% have been obtained on 60- to 90-day non-returns to 1st services.

This diluter of 40% egg yolk and 60% sodium citrate has been compared with a 20% egg yolk and 80% sodium citrate diluter and with the liquid Ortho semen diluter. In the College herd, based on actual calvings of 55 females, the following conception rates to 1st services were observed: 40% egg yolk showed 68%, 20% egg yolk showed 75%, and Ortho showed 60%. This work was followed by field trials in the county associations. Based on 65-day non-returns to 537 1st services, 40% egg yolk gave 66%, 20% egg yolk gave 61% and Ortho gave 62%.

## MANUFACTURING SECTION

**M1. Ion exchange as a means of varying the salt constituents of milk.** H. S. HALLER AND A. G. MORIN, Bureau of Dairy Industry, U.S.D.A.

Milk can be modified by treatment with various types of organic ion exchange materials to produce certain desired characteristics. The type and extent of modification can be controlled at will by using the proper exchangers or combination of exchangers and by the proper regeneration of these exchangers.

An organic cation exchanger regenerated to a 99:1 Na-H ratio produced a soft curd milk of normal pH with one treatment. The amounts of Ca and other cations removed depended on the time of treatment and the milk-to-exchanger ratio. This milk retained all of its citrates, phosphates and chlorides.

Treatment with a cation exchanger regenerated to a 93:7 Na-H ratio, followed by treatment with an organic anion exchanger, removed up to approximately 20% of the citrates, phosphates and chlorides in addition to Ca and other cations. The ratio of cations to anions removed was controlled by using a cation exchanger regenerated with a Na percentage ranging between 93-99, followed by treatment with an anion exchanger.

Treatment with a strong anion exchanger, followed by careful treatment with a small amount of cation exchanger regenerated 100% in the hydrogen cycle, removed citrates and chlorides and small amounts of Ca and other cations, but no phosphates.

**M2. Isolation of the non-casein proteins of milk.** A. R. KEMP, B. C. JOHNSON AND A. M. SWANSON, Univ. of Wisconsin

Many properties of milk have been attributed to the non-casein proteins. Only limited quantities of certain of these proteins have been isolated for study.

In the present study 4 fractions of non-casein proteins are obtained by control of salt concentration, pH and temperature. Casein is precipitated by acid at pH 4.5, 10° C.; non-casein proteins are isolated as follows: Fraction 1—salt saturation at pH 6.0-6.2, 2° C. The precipitate is allowed to settle, supernatant siphoned off and the precipitate washed 3 times with saturated salt water. Fraction 2—the precipitate formed with 14 g. salt/100 ml. solution at pH 3.5 or lower and temperatures up to 25° C. Fraction 3—the filtrate from fraction 2.

Curves were obtained of protein precipitation by changes in pH from 7.0 to 2.0 in a solution saturated with salt and of protein precipitation by changes in salt concentration at pH 2.0. Fraction 1 after purification was found not to precipitate in saturated salt solution at pH 6.0-6.2 but did precipitate at pH 5.0; it was completely precipitated at pH 3.5 and 14 g. salt/100ml. solution. Fraction 2 contains at least 2 components, 1 insoluble in distilled water and 1 that is soluble.

**M3. A detailed study of the non-protein nitrogen fractions in milk.** K. M. SHAHANI AND H. H. SOMMER, Univ. of Wisconsin

Non-protein-nitrogen fractions like urea, ammonia, creatinine, creatine, uric acid and  $\alpha$ -amino nitrogen in milk have not been studied as thoroughly as the protein fractions. It is of importance to know what changes take place in the non-protein-nitrogen fractions of milk when the milk is subjected to different plant treatments like pasteurization, homogenization, etc. A detailed investigation with reference to these and also with reference to the effects of feeding has been made.

Total nitrogen, casein, albumin, globulin, proteoses, peptones and non-protein-nitrogen were determined by methods suggested by Menefee, Overman and Tracy with slight modifications. Urea and ammonia were determined according to Perkins on protein-free filtrate by use of urease, and the ammonia was distilled into boric acid, using MgO. Creatine and creatinine were determined colorimetrically, using picric acid and NaOH to develop the color in the protein- and lactose-free filtrate. Creatine first was hydrolysed by acid in an autoclave. Uric acid was determined on the protein- and fat-free filtrate by treating it with NaCN and arsenophosphotungstic acid which resulted in blue color which was compared with a standard. Alpha-amino-nitrogen was determined on milk serum prepared by use of acetic acid and copper acetate. The milk serum was concentrated and Van Slyke determinations were made.

Following data represent the average of 10 composite milk samples as compared with the data of other workers:

	(mg./100ml.) (Present results)	(mg./100ml.) (From literature)
Total nitrogen	474.07	468.10 <sup>a</sup>
Casein N	371.96	375.20 <sup>a</sup>
Aubumin N	37.86	48.90 <sup>a</sup>
Globulin N	24.12	17.80 <sup>a</sup>
Proteoses and peptones	16.25	15.30 <sup>a</sup>
Non-protein-nitrogen	23.88	22.97
Urea	8.60	9.33
Ammonia	0.76	0.42 <sup>b</sup>
Creatinine	0.46	1.42
Creatine	3.91	2.37
Uric acid	2.26	1.53
Alpha-amino-nitrogen	3.86	4.24
Unaccounted	4.21	4.07

<sup>a</sup> Values reported by Menefee *et al.*

<sup>b</sup> Perkins

Rest by Denis and Minot.

**M4. A colorimetric determination of lipase.** (A preliminary report.) GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U.S.D.A.

A rapid method of determining lipase based on the splitting of  $\alpha$ -naphthyl esters of the fatty acids is described. The principal substrate is  $\alpha$ -naphthyl acetate. Other substrates and their uses are discussed. The color (deep purple) is produced when 2,6-dibromoquinone chloroimine (B.Q.C.) combines with  $\alpha$ -naphthol liberated by the lipases. The effect of time, temperature, pH and concentration of the substrate is discussed. It has been found possible to detect the presence of 1 lb. of raw milk in 2,000 lb. of heated milk. The determination may be made in 1 hr. Where the concentration of lipase is low, the reaction time may be increased without appreciable error caused by acid formation or other changes. The use of the method in determining the specificity of lipases is discussed.

**M5. Nephelometric determination of fat in non-fat dry milk.** BURDET HEINEMANN, E. J. BALDI AND O. B. PARKER, Producers Creamery Co., Springfield, Mo.

The Mojonner method is the only published method suitable for the determination of fat in non-fat dry milk. The following procedure is the result of attempts to develop a less time-consuming method.

Four g. of NFDM are reconstituted and made to 100 ml. with water. Five ml. are placed in an oil-sample bottle and the following reagents added with mixing: 5 ml. H<sub>2</sub>O, 1.5 ml. NH<sub>4</sub>OH, 10 ml. alcohol, 25 ml. ethyl and 25 ml. petroleum ethers. Centrifuge 30 sec. and transfer 25 ml. of ether layer to aluminum dish. Evaporate to complete dryness on 135° C. hot plate then float dish on water at 45° C. Add 10 ml. dioxan at 45° C. and swirl 60 sec. Pipette 5 ml. dioxan to 100 ml. pH 2 buffer (Clark and Lubs) containing 0.1% low viscosity carboxymethyl cellulose at 25° C., using a magnetic stirrer at a constant speed. Read 4 min. later in Coleman Universal Model 11 equipped with Nephelometric attachment, setting blank at zero. The curve is obtained by extracting fat from skimmilk, dissolving known quantities in dioxan and adding 5 ml. of the various concentrations (0.05–0.2 mg./ml.) to 100 ml. of buffer under standard conditions.

A complete determination may be made in 20 min. Forty-two comparisons between individual tests by the standard Mojonner procedure and the above method resulted in a standard deviation of  $\pm 0.09\%$  fat. The range of samples tested was from 0.48 to 1.80% fat.

**M6. The action of mineral-ion exchange resins on certain milk constituents.** CHARLES W. GEHRKE, Univ. of Missouri, AND EMORY F. ALMY, Ohio State Univ.

When 0.100 *N* solutions of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{K}^{+}$  and  $\text{Na}^{+}$  ions as the chlorides are subjected singly to cation exchange using Zeo-Karb-II, the order of adsorption was  $\text{Ca}^{++} > \text{Mg}^{++} > \text{K}^{+} > \text{Na}^{+}$  ion. A quantitative relationship existed between the adsorption of the metallic ions from the solution and the hydrogen ions released from the exchanger to the effluent.

The order of adsorption of the metallic ions mentioned above in binary, ternary and quaternary solutions totaling 0.100 *N* was the same as that found for the removal of the cations when they were singly passed through the exchanger.

A variation in the concentration of the constituent ions of the binary solutions did not cause a change in the order of adsorption of the cations studied.

The cations present in simple solutions and in more complex solutions as well (such as milk and whey) which are least adsorbed by the exchanger are found to be removed during the first part of the exchange run, then released later by the regeneration effect of other cations which are preferentially adsorbed.

Ca ions were found to be more effectively adsorbed from a solution containing citric acid than from a solution containing HCl.

The initial removals to the break-through-point for solutions in which the constituent ions have varying concentrations are dependent upon the concentration, type and valence of the component ions of the solution.

**M7. Preliminary observations on the electrophoresis study of the proteins in skimmilk.** W. L. SLATTER AND Q. VAN WINKLE, Ohio State Univ.

In this study of the components of skimmilk, the electrophoresis apparatus of Tiselius, as modified by Longsworth and Mac Innes, was used. The boundary positions were followed with the Schlieren optical system. Fresh skimmilk was examined at pH 3.1, 4.1, 5.4, 6.6 and 8.5, at ionic strengths of 0.02, 0.10 and 0.50.

At pH 6.6, the descending boundaries indicated that 4 components were present, whereas the ascending boundaries showed only 3 components. The 4th component was indicated by a boundary that moved only slightly and may be wholly or partly an epsilon boundary effect. At this pH, patterns of the descending boundaries were quite different from those of the ascending boundaries, which suggest an interaction between proteins. This interaction between components

apparently was at its maximum at pH 6.6.

As the ionic strength was reduced from 0.50 to 0.02, a tendency for the skimmilk to break-up into more components was observed. The 2 components which migrated the most rapidly sometimes were difficult to distinguish at an ionic strength of 0.50, especially at pH 6.6.

When skimmilk was heated to 65, 75 and 85° C. for 30 min. the electrophoretic patterns underwent considerable change. One component disappeared completely from the pattern when the milk was heated to 75 and 85° C. for 30 min.

**M8. State of solution of the naturally occurring salts in milk.** INDRAPAL S. VERMA AND H. H. SOMMER, Univ. of Wisconsin

The distribution of Ca, Mg, P and citric acid in milk has been studied to determine total amounts and the soluble fractions. The soluble amounts of these constituents were determined by analyzing whey prepared by use of rennet. All 4 constituents were studied on the same sample simultaneously. Only 85% of the total citric acid present in milk exists in soluble form. Analyses of 15 such samples gave the following results (mg./100 ml.):

	Total	Soluble
Ca	132.1	51.8
Mg	10.8	7.9
P	95.9	36.3
Citric acid	154.2	141.6

The influence of pasteurization and cool aging (at 45° for 24 hr.) also was studied on these constituents. There was a decrease in the amount of soluble calcium on pasteurization which tended to come to normal on cool aging and at the end of 24 hr. the amount of soluble calcium in milk was higher than that contained in raw milk. A decrease in the soluble calcium also was accompanied by a simultaneous decrease in the pH and vice versa. These changes in the pH and the phenomenon of retardation of rennet coagulability of milk are explained in terms of precipitation of tribasic Ca and Mg phosphates. The results were supported by the experiments run to study the influence of added Ca and phosphates on the distribution of salts. It finally was confirmed by the behavior of the salts in a solution prepared from pure salts to resemble the composition of salts in milk and at the reaction of normal milk.

**M9. Studies on oxidized milk fat.** MARK KEENEY AND F. J. DOAN, Pennsylvania State College

Oxidized milk fat was vacuum-distilled to recover the volatile compounds. Major attention

was given to the ether-soluble, neutral fraction, of the distillate. This fraction is a potent flavor concentrate of compounds characteristic of oxidized milk fat. Further separation of the neutral fraction by fractional distillation and reaction with carbonyl reagents indicate that carbonyl compounds are responsible for the predominating odor and flavor of oxidized milk fat. The carbonyl fractions are capable of imparting an oxidized flavor to several million parts of milk.

The identification of these compounds is being attempted by qualitative tests and preparation of derivatives. The hydrazone of a 9-carbon carbonyl compound has been prepared from the neutral fraction and indications are that it is derived from an unsaturated ketone. The hydrazone of a 3-carbon carbonyl compound containing a non-carbonyl oxygen atom also has been prepared. This may be a derivative of lactic aldehyde. Proof of this assumption, however, awaits preparation of an authentic derivative.

The ether-soluble acidic fraction of the milk fat distillate browns readily. The odor, when freshly isolated, is reminiscent of a 6- to 8-carbon acid. In a few days at room temperature it assumes a coconut-like odor. This fraction also reacts with carbonyl reagents. These observations suggest that keto acids are present which slowly dehydrate to lactones.

**M10. The enzymatic hydrolysis of lactose in dairy products and its determination.** FRANK E. POTTER, Agricultural Research Administration, U.S.D.A.

A colorimetric method has been adapted to the determination of glucose and galactose in the presence of lactose. It is a measurement of the blue color produced by the sugars with  $(\text{NH}_4)_2\text{MoO}_4$  and  $\text{KH}_2\text{PO}_4$  when heated. The method is based on difference in rate of the color production by the sugars.

The transmittancy at a wavelength of 640 m $\mu$  for 1 mg. of lactose in 25 ml. of solution is approximately 99%. An equimolecular mixture of glucose and galactose under similar conditions exhibits 79% transmittancy. When 0.1–5.0 g. of a glucose-galactose mixture was added/100 g. of milk, the average value found for 14 samples was 98.70% of the amount added.

An enzyme capable of hydrolyzing the lactose in whole or concentrated milk products has been supplied by a commercial chemical company. The optimum temperature of enzyme activity is approximately 40° C., but appreciable hydrolysis is obtained at lower temperatures. The enzyme added to condensed skim milk at the rate of 0.5% of the lactose content produced 23% hydrolysis in 5 d. at 4° C. No change in titratable acidity or pH occurred. A comparable hydrolysis can be

produced at 30° C. in a few hours.

The hydrolyzed products may be used to produce ice cream of high serum solids content that will not become sandy during storage.

**M11. Studies on the water-insoluble acids of butter.** F. J. BABEL, Purdue Univ.

Lots of cream testing approximately 35% butterfat were separated from fresh milk. Each lot was divided into 2 portions and held at 55 and 75° F. A sample was taken from each portion daily for 7 d. and at 10 d., neutralized to pH 6.8 with a soda-type neutralizer and churned. The resulting samples of butter were analyzed for water-insoluble acids by the method of Hillig (J. Assoc. Off. Agr. Chem. 30: 575–582. 1947).

The cream held at 55° F. did not increase in acidity as rapidly as that held at 75° F., nor did it show as rapid development of mold. The % titrable acidity of the samples held at 55 or 75° F. was not materially different after 3 d., although the samples held at 55° F. were always slightly lower until the cream was held for 10 d. The water-insoluble acid content of the butter made from fresh cream was about 240 mg./100 g. fat. The water-insoluble acid content of the butters did not increase materially with an increase in age of the cream at either temperature of holding. There was no significant difference in the water-insoluble acid content of butters made from cream held at 55 or 75° F.

Preliminary data indicate that when cream sours rather rapidly, the lipases are inhibited because of the unfavorable pH. Cream having a clean acid flavor produced butter with a relatively low water-insoluble acid content, while cream having a low acidity but evidence of putrefactive types of microorganisms usually produced butter with a high water-insoluble acid content.

**M12. The lactometer as an instrument for determining added water in milk.** O. M. YSTGAARD, P. G. HOMEYER AND E. W. BIRD, Iowa State College

Limiting formulæ based on changes in serum solids have been proposed for determining added water, c.f.  $\frac{S_1 - S_2}{S_1} \times 100$ , in which  $S_1$  is the se-

rum solids in the non-watered milk and  $S_2$  those of the sample observed. The legal minimum generally is used as  $S_1$  and obviously invalidates the calculation.

In this study, the Babcock fat test was determined, and the corresponding serum solids from the table presented by Jacobson (J. Dairy Sci., 19: 170–176. 1936.) was used as  $S_1$ .  $S_2$  was determined from the Babcock fat test and the lactometer reading. The lactometric procedure

of Sharp and Hart (J. Dairy Sci., 19: 683-695. 1936.) was employed; the calculations were made by the following modified Herrington (J. Dairy Sci., 29: 87-89. 1946.) formula:

$$I. \% T.S. = 1.2537 F + \frac{268.0 (L + 3)}{(L + 1000)} - 0.15$$

Total solids by formula I ( $T.S._I$ ) agreed well with total solids by the Mojonnier method ( $T.S._M$ ). The regression equation relating the two is:

$$II. \% T.S._I = 0.9835 T.S._M + 0.2248$$

The correlation coefficient between the 2 methods is 0.9958.

The percentage added water was calculated by formula III ( $\% H_2O_{III}$ ) and from freezing point data ( $\% H_2O_{FP}$ ).

$$III. \% H_2O_{III} = \frac{(S_1 - S_2)}{S_1} 100 - 0.2$$

The regression equation relating the percentages of water calculated by the 2 methods is:

$$IV. \% H_2O_{III} = 1.019 \% H_2O_{FP} + 0.0173$$

The correlation coefficient between the 2 methods is 0.9535.

#### M13. Rapid salting of brick cheese.<sup>1</sup> H. J. BUYENS AND W. V. PRICE, Univ. of Wisconsin

The brine salting of brick cheese in factory practice usually begins after the curd has drained in hoops for 18 hr. In this experiment the cheese was brine salted after only 6 hr. in metal hoops and the results were compared with normal factory practice.

Salt, moisture and pH measurements were made on a composite sample and on 4 layers beginning with the rind and extending to the center. These tests were made immediately after salting and 4 wk. later at scoring. Tests of composite samples of cheese salted in 22% NaCl brine for 24 hr. showed an average of 1.73% salt in the lots drained for 6 hr. and 1.09% salt in the lots drained 18 hr. This higher trend in salt was most apparent in the outer layers.

The early salting treatment caused a reduction in the amount and firmness of rind formed; this was accompanied by faster "smear" development, smaller moisture loss and higher yield. The quality of the cheese equalled or was slightly better than that of the normal cheese. The early salting treatment makes it possible to hasten the primary changes in curing by more than 24 hr.

<sup>1</sup> Cooperative RMA project with the Bureau of Dairy Industry, U.S.D.A.

#### M14. Pasteurization of milk for Italian cheese

curd.<sup>1</sup> J. C. MARQUARDT, New York State Dept. of Agriculture and Markets, Albany

The curd commonly is made from milk containing 1, 2, 3 and more than 3% fat. A procedure survey indicated that deviations are not sufficient to change the pH from the range 6.2-6.4 on the fresh curd. Some of the curd is used without further processing. The curd also is shaped after a hot water treatment; the pH range is 5.3-5.5 at this point.

A plant was selected with a flash heater approved by the N. Y. State Dept. of Health. A double check on 48 samples by the State Health Department and the N. Y. City Dept. of Health showed that the milk was pasteurized properly. Curds made from properly-pasteurized milk were superior in flavor to raw-milk curds. There was no problem in molding the cheese made from properly pasteurized milk, although the aging period was increased.

Yields, aging control and a simple procedure for following the aging are being investigated.

<sup>1</sup> Approved for presentation by the New York State Dept. of Agriculture and Markets.

#### M15. The influence of ultrasonic sound waves on cheese ripening. W. C. WINDER, A. M. SWANSON AND W. V. PRICE, Univ. of Wisconsin

The application of ultrasonic sound waves to cheddar cheese on a laboratory scale was shown to accelerate the ripening process. Cheese curd either in the whey before dipping or at 1 day of age after removal from the press was treated ultrasonically. Improved cheese consistently was produced when conditions of cheese volume, duration of exposure and wave transmitting media were controlled properly. Measurements of water-soluble nitrogen showed that protein hydrolysis progressed much more rapidly in the ultrasonically-treated cheese than in the untreated cheese. Amino acid assays indicated a more complete protein hydrolysis in the treated cheese; glutamic acid and leucine, which are associated with cheese flavor, were found in increased amounts. Ultrasonic treatment of cheese altered the normal sequence of bacterial development and induced the development of abnormally high populations of streptococci, micrococci and lactobacilli. In simple-system studies the action of ultrasonic sound waves was shown to increase the pH, split lactates and citrates, and produce flavor compounds from organic acids, salts and butterfat.

#### M16. Observations on an exudate from cheddar cheese. V. L. ZEHREN AND A. M. SWANSON, Univ. of Wisconsin

An amber colored exudate occasionally is ob-



served on the surface of cheese during curing. This exuded material is observed more often on cheese cured at higher temperatures. The exudate, when it first appears, is quite fluid, but on exposure to air it becomes viscous and sticky. Rarely is it possible to accumulate any quantity of this material for analysis. A sample of about 3 oz. was obtained from the cheese and shelves of a lot of cheese which had been cured at 60° F.

This is a preliminary report on the analysis of the material exuded from this cheese. The exudate contained 0.6% fat by the Mojonnier method. It contained 65.9% total solids and 34.1% moisture. The ash content was 14.9% of the total solids. The total nitrogen content was 6.5%, of which little was protein nitrogen, as the proteose and peptone nitrogen was 5.7%. The  $\alpha$ -amino nitrogen was 2.5%.

**M17. The forced-drying of cheddar cheese prior to paraffining.** D. M. IRVINE AND W. V. PRICE, Univ. of Wisconsin

The drying of cheese before paraffining is a space-, labor- and time-consuming operation. The cheese must be dry before paraffin can be applied.

Daisies from the same vat were subjected to normal and forced-drying. Forced-drying consisted of holding the cheese in an air blast at 60–120° F. for periods of from 4–14 hr. The heated air moved over the cheese at the rate of approximately 1,450 ft.<sup>3</sup>/min.

Cheese held in the air blast at 80° F. for 8 hr. plus 1 hr. at 60° F. lost less weight than the controls. This was the best treatment revealed in this study. Cheese dried at temperatures above 100° F. lost less weight during forced-drying but more during storage than the controls. The quality of the forced-dried cheese was slightly better. Curdiness was retained longer in the force-dried lots. Defects in finish of cheese dried above 100° F. were excessive mold growth, misshapen cheese and a slight exudation of a whey-like fluid.

The 0.25-in. rind layer of the cheese dried at 80° F. averaged 1.8–2.5% more moisture than the controls sampled identically. Weight lost before paraffining averaged 0.915% in 10 lots forced-dried at 80° F., while the comparable normally-dried cheese lost 2.16%.

It seems possible that the time from pressing to paraffining cheddar cheese can be safely reduced to 9 hr.

**M18. The free amino acids of foreign type cheese.** F. V. KOSIKOWSKY AND A. C. DAHLBERG, Cornell Univ.

A number of commercial foreign type cheese

were analyzed for their free amino acids and other water soluble protein decomposition products, using two dimensional paper partition chromatography. A rough quantitative method utilizing color standards was applied to the analyses of these cheeses. Among the cheese types studied were swiss, roquefort, limburgier, leiderskrantz, camembert and Italian.

The free amino acid concentrations were found to be highly variable within cheese types, undoubtedly due to the different ages and past histories of the commercial cheese. Most of the cheese showed about 14–17 free nitrogenous compounds. An example of an extreme case was a commercial muenster cheese which showed within the limits of sensitivity of this method the presence only of small amounts of 2 free amino acids, glutamic acid and aspartic acid.

It was difficult to characterize foreign cheese types from commercial sources by their free amino acid patterns because of the variability of these patterns within types. However, preliminary observations indicate that the average swiss cheese is associated with higher free proline concentrations and the average roquefort cheese with higher free tyrosine concentrations than the other cheeses studied.

**M19. The methyl ketones of blue cheese.** STUART PATTON, Pennsylvania State College

Blue cheese (roquefort type) has been studied relative to the specific methyl ketones present. The ketones first were separated from the cheese by steam distillation and then, with the exception of acetone, removed from the distillate by ether extraction. Removal of solvent from the extract, followed by distillation of the residue, yielded fractions which were found to be relatively pure 2-pentanone, 2-heptanone and 2-nonanone. These compounds were identified by comparison of data on boiling points, refractive indices and results in certain qualitative tests with those of the known ketones. Identification was confirmed by performance of mixed melting points on derivatives prepared from the unknown compounds and the known ketones. A considerable quantity of material boiling above 200° C. was obtained during the fractional distillation. The column employed did not appear to be effective in fractionating these compounds; however, the results of suitable qualitative tests demonstrated that this material contained traces of additional methyl ketones. Acetone was identified in the extracted steam distillate, the indications being that the ether extraction procedure was ineffectual in removing it therefrom.

The presence of the type ketones identified in these experiments is explainable on the basis of

$\beta$ -oxidation of milk fat acids. The potent odor and biting taste qualities of these ketones are closely related to the typical flavor of blue cheese. Additional work is in progress concerning the mechanism of the mold action and quantities of ketones produced during the curing of blue cheese.

**M20. Tyramine production in cheese and in various bacterial cultures.** JOHN A. HUPFER, JR., GEORGE P. SANDERS AND RALPH P. TITSLER, Bureau of Dairy Industry, U.S.D.A.

Tyramine analyses were run on pairs of cheddar cheese, one made from raw milk and the other from pasteurized milk. The cheese made from raw milk generally contained more tyramine than that made from pasteurized milk and was poorer in quality. The use of poor-quality raw milk resulted in increased tyramine content and poorer-quality cheese.

Pasteurized-milk cheddar cheese was made to compare lactic starter with lactic plus enterococcus. Enterococcus starter greatly increased tyramine production, but did not affect the average quality of the cheese.

Sixty strains of bacteria, representing 28 species and 10 genera, were tested for production of tyramine in milk containing added tyrosine. Tyramine values in  $\mu\text{g./ml.}$  of culture were: *Streptococcus durans*, *S. liquefaciens* and *S. zymogenes*, 228-280; *S. faecalis*, 24-188; *S. thermophilus*, 28; *Microbacterium lacticum*, 48; *Lactobacillus acidophilus*, 0-40; *Leuconostoc citrovorum*, *Leuc. dextranicum*, *Leuc. mesenteroides*, 25-18; and *L. brevis*, *L. fermenti* and *L. buchneri*, 24-112. The following gave values of 0-12  $\mu\text{g./ml.}$ : *S. lactis*, *S. cremoris*, a lactic starter, *Escherichia coli*, *Acrobacter aerogenes*, *Bacillus cereus*, *B. megatherium*, a proteolytic coccus, *Bacterium linens*, *L. bulgaricus*, *L. lactis*, *L. casei*, *L. plantarum*, *Propionibacterium shermanii* and *P. freudenreichii*.

**M21. The effect of penicillin and streptomycin on swiss cheese starters.** R. E. HARGROVE, H. E. WALTER, J. P. MALKAMES, JR., AND K. T. MASKELL, Bureau of Dairy Industry, U.S.D.A.

During swiss cheese investigations, inhibition of starters was noted due to penicillin and streptomycin in the milk supply. A study was undertaken to determine: (a) Percentage of total antibiotics shed in milk by a treated cow; (b) antibiotic sensitivity of cheese starter strains; (c) heat tolerance of antibiotics in milk; (d) inactivation of penicillin by Penase; and (e) the possibility of developing resistant starter strains.

Milk obtained during treatment contained

from 26 to 49% of the injected penicillin and from 39 to 58% of the streptomycin. Only 0.04% of the penicillin and no streptomycin remained in the second milking after treatment. The following starter strains showed marked inhibition: *Streptococcus thermophilus* by 0.01 unit penicillin and by 5  $\mu\text{g.}$  streptomycin/ml.; *Lactobacillus bulgaricus* by 0.1 unit penicillin and by 1  $\mu\text{g.}$  streptomycin/ml.; *Propionibacterium shermanii* by 0.1 unit penicillin and by 5  $\mu\text{g.}$  streptomycin/ml. Neither penicillin nor streptomycin was destroyed by pasteurization. Streptomycin was drastically reduced upon autoclaving and steaming, while penicillin was more stable. One ml. of Difco Penase concentrate was required/liter of milk to inactivate 0.5 unit penicillin/ml. *L. bulgaricus* and *S. thermophilus* strains were developed which were resistant to 3 units of penicillin and to 500  $\mu\text{g.}$  of streptomycin/ml., and *P. shermanii* strains to 1 unit of penicillin and to 200  $\mu\text{g.}$  streptomycin/ml.

**M22. Observations on a gelatinous curd type of spoilage of cottage cheese.** R. B. PARKER, V. N. SMITH AND P. R. ELLIKER, Oregon Agr. Expt. Station

A study was made of a cottage cheese defect in which the curd particles developed a heavy, gelatinous, translucent coating. The defect normally appeared after 2-4 d. storage at 45-50° F. Creaming of the curd appeared to stimulate development of the defect.

Large numbers of an *Alcaligenes* and a *Pseudomonas* species were isolated from both defective curd and the plant water supply. Pure cultures of these organisms produced the same type of defect experimentally. Studies indicated that a pH of 5.0 or lower in the curd mass or hypochlorite treatment of the water supply or both were necessary to prevent development of the defect.

**M23. Low cost and small scale methods for concentrating whey for feed.** A. H. STEVENS, Bureau of Dairy Industry, U.S.D.A.

Two methods by which the small cheese factory may concentrate whey without the use of vacuum pan were investigated. Whey was evaporated (a) by means of a submerged combustion unit and (b) by spraying it into heated air in a collector similar to those used on a cyclone spray drier.

The submerged combustion unit consisted of auxiliary equipment for supplying air and natural gas to the burner in the correct ratio and at suitable pressure to maintain combustion under the surface of the liquid. Foaming, caused by the turbulent combustion of the air-gas mixture un-

der the surface of the whey, was suppressed by use of a translucent silicone compound. One ft.<sup>3</sup> of methane gas evaporated 0.69 lb. of water. The whey could be concentrated to about 20% solids, but it had a strongly caramelized color, odor and flavor.

Whey preheated to temperatures above 212° F. was sprayed into a cyclone of heated air during concentration in the cyclone spray evaporator. Results were obtained by use of cones of 2 sizes and with 2 types of sprays during operation at various whey and air temperatures. Whey was continuously concentrated to about 33% solids in this kind of equipment. The physical characteristics of this concentrate compared favorably with those of whey condensed under vacuum.

**M24. The utilization of whey in the microbiological synthesis of riboflavin.** ABRAHAM LEVITON AND EARLE O. WHITTIER, Bureau of Dairy Industry, U.S.D.A.

Whey is an excellent source of nitrogen and accessory factors for the microbiological synthesis of riboflavin by means of the organism *Ashbya gossypii*. However, lactose is not utilized. By means of mild hydrolytic treatment, which may be incorporated in the sterilization step, yields in excess of 200 mg./l. of whey may be realized on a laboratory scale. The non-heat-coagulable nitrogenous material of whey is the most readily utilized portion. The heat-coagulable portion, on the other hand, contributes but little to riboflavin yield. Maximum efficiency is obtained in dilute whey solutions. A dilution of 1 part treated whey with 1 part water represents a desirable proportion. The use of untreated whey instead of water as a diluent promotes increased yields.

The culture as originally received contained a mixture of cells, some of which were non-productive. By a selective process and over a long period of time, these were eliminated to yield a culture with stable characteristics.

Oxygenation promotes a more rapid synthesis, but does not result in an increase in final yield. In aeration experiments, the use of milk fat and ethylene glycol as foam depressants resulted in poor yields. The use of silicone anti-foaming compounds gave much better results.

**M25. Strains of *Streptococcus faecalis* present in a starter used in the manufacture of cheddar cheese.** A. C. DAHLBERG AND F. V. KOSIKOWSKY, Cornell Univ.

In 1946, 2 colonies were selected from azide-penicillin agar plates that proved to be *Streptococcus faecalis* which rapidly produced acid in

milk. One starter for use in the manufacture of cheddar cheese was made from them and it has been carried in milk pasteurized at about 200° F. for 1 hr., using a 1% inoculation and 90° F. incubation. The frequency of transfer has been irregular but usually 3 times/week.

In 1949 the question arose as to the purity of the starter. It was plated on tomato juice-yeast extract-tryptone-beef extract-milk agar, and 50 colonies were selected. They all were *S. faecalis* as they were Gram positive streptococci that grew at 10 and 45° C. and in 6.5% salt broth, in penicillin broth (100 units/l.) and in 0.1% methylene blue milk. All cultures reduced and curdled milk and fermented glucose, glycerol and maltose. They decarboxylated tyrosine, hydrolyzed sodium hippurate and showed a green zone on horse blood agar. None of the cultures fermented arabinose, inulin and raffinose.

The predominating strain of bacterium did not ferment mannitol, sorbitol or sucrose and a 1% inoculation reduced and curdled milk in 18 hr. A second strain reduced and curdled milk in 18-48 hr., and it fermented mannitol, sorbitol and sucrose. It should be obvious that, since 2 colonies which curdled milk rapidly were used for the initial starter, both of these strains may have been present. However, a 3rd strain fermented mannitol, sorbitol and sucrose, but it required about 4 d. to curdle milk. This strain could not have been present in the original starter except as an accidental contamination. It is more reasonable to assume that this 3rd strain was a variant of the second, as it is known that *S. faecalis* sometimes loses its ability to ferment lactose.

**M26. Bacterial studies of the high-temperature short-time pasteurization of ice cream mix.** F. W. BARBER AND H. P. HODES, National Dairy Research Laboratories, Inc., Oakdale, N. Y.

Raw ice cream mix and ice cream mix inoculated with an organism of known heat resistance each were pasteurized in an experimental high-temperature, short-time pasteurizer at 165, 175, 185 and 190° F. for 25 sec. holding time and at 190, 210, 240 and 260° F. for 1.4 sec. holding time. The pasteurization efficiency of the experimental pasteurizer was evaluated by the determination of the per cent destruction of the normal bacterial flora and the heat-resistant test organism and by calculation of the total lethal effect of each pasteurization treatment. High-temperature, short-time pasteurization at 175° F. or higher for 25 sec. holding time and at 190° F. or higher for 1.4 sec. holding time give results that are comparable to the presently accepted pasteurization standard for ice cream mix.

**M27. The influence of pH on proliferation of the lactic streptococcus bacteriophage.** W. W. OVERCAST, F. E. NELSON AND C. E. PARMELEE, Iowa Agr. Expt. Station

Several strains of the lactic streptococci and their homologous bacteriophage strains were studied to determine the influence of pH on phage proliferation. From pH 5.2-9.4 litmus milk was used as the substratum; below pH 5.2, a vegetable juice-peptone broth was used. The pH of the substratum was maintained by frequent electrometric pH determinations and addition of 1N NaOH when necessary. The numbers of organisms were determined at intervals by plate counts on TGEM agar. The phages were enumerated by the three-tube limiting dilution technique, using litmus milk fortified with vegetable juice as the medium.

Most strains of phage showed a marked reduction in the rate of increase at pH levels below 5.4; however, some strains were able to increase at pH 4.8. The lag phase at the low pH levels sometimes was prolonged as much as 12-18 hr. When some strains of phage and organism were increasing in milk and the reaction suddenly was lowered to pH 5.2-5.3 by adding lactic acid, phage increase virtually ceased, while acid production continued. When heavy organism inoculations and small numbers of phage particles were used, the rapid lowering of the pH below the critical level for phage proliferation usually prevented occurrence of mass lysis.

With some combinations, phage proliferation virtually was stopped at pH 7.6, even though the organisms increased at a rapid rate. With others, the higher pH prolonged the lag phase but did not prevent later phage increase. Only one phage strain tolerated the high pH as well as the organisms did and this strain showed a definite increase in 24 hr. at pH 9.4.

**M28. Changes in bacteriophage and sensitive organism populations in a commercial mixed culture.** C. E. PARMELEE, F. E. NELSON AND W. W. OVERCAST, Iowa Agr. Expt. Station

Several attempts to isolate bacteriophage from slow vats of milk set with a commercial mixed strain culture had failed when the mixed-strain culture was used as the test culture. The use of single colony isolations as test cultures resulted in the isolation of several phage types.

Transfers from the original culture had been frozen at various times over a 2-yr. period. The sensitivity to 3 phage types of isolates made from first transfers of the frozen cultures varied greatly. Cell-free filtrates made from the first transfer from frozen cultures indicated that the culture carried bacteriophage at different times.

At one time 83% of the organisms in the culture were sensitive to one phage type. Incubation of 4 ml. (ca.  $1 \times 10^9$  cells) of this culture and 20 phage particles in 400 ml. of steamed skim milk for 16 hr. at 21° C. resulted in normal coagulation of the milk, complete shift of the organisms to strains not sensitive to the phage added and a titer of  $6 \times 10^9$  phage particles/ml. in the cell-free whey. Successive transfers of this culture serially diluted the phage until it disappeared. The first transfer of a new culture from the original supplier contained a phage of somewhat similar activity pattern.

**M29. A study on psychrophilic bacteria in market milk.** F. A. ROGICK AND L. H. BURGWARD, Ohio State Univ.

A study on psychrophiles and their relationship to mesophiles was made on raw and pasteurized milk supplies. Both H.T.S.T. and L.T.L.T. systems were studied over a period of 1 yr.

The experimental data showed that the psychrophiles were not thermoduric and were mostly cocci or non-sporeforming bacilli, inert or acid-producing, facultative rather than true psychrophiles. In every instance the mesophilic counts were higher than the psychrophilic counts in the fresh milk. The mesophilic and psychrophilic counts both showed a definite increase in all samples at the end of 1 wk. of storage in a refrigerator. At this time, however, the psychrophilic count invariably was higher than the mesophilic count.

No psychrophiles ever were found in 4.1 ml. of fresh pasteurized milk taken from the vat (L.T.L.T) or at the cooler (HTST), but at the end of 1 wk. of storage an average of slightly over 50,000/ml. was found. It is thought that some of the mesophiles develop psychrophilic properties. The psychrophilic counts in the fresh, pasteurized bottled milk always were low and attributed to contamination after pasteurization from water used in rinsing equipment, from the equipment itself and, less frequently, from the bottles.

The initial bacterial count of the fresh raw milk was not a true index for determining the further development of psychrophiles.

There was no appreciable difference between the spring, fall and winter mesophilic and psychrophilic counts, but the respective counts were all higher in the summer.

**M30. Effects of storage on penicillin in dairy products.** W. A. KRIENKE AND E. L. FOUTS, Florida Agr. Expt. Station

*Pasteurized milk:* When added at the rate of 0.25 unit/ml. of milk prior to pasteurization,

penicillin retained its property of completely inhibiting acid production by a lactic starter (*Streptococcus lactis*) through 10 d. of refrigerated storage as was evidenced by a titratable acidity, after 9 hr. of incubation at 95° F., of 0.19% as compared to 0.63% for some of the same milk containing no penicillin, both inoculated with 3% active culture.

**Evaporated milk:** Although penicillin had been added to whole milk at the rate of 1.0 unit/ml. of milk prior to heat treatment and concentration, there was only a slight effect of penicillin in the evaporated milk immediately after sterilization on acid production of a portion cultured (3%) and incubated at 95° F. for 7 hr. After 9 d. of storage at room temperature its effect on acid production was practically nil.

**Condensed whole milk:** A portion of the concentrated milk prepared for the evaporated series was placed into refrigerated storage soon after removal from the pan. Another similar portion was mixed with an equal volume of control ("drug-free") and refrigerated also. At both concentrations of penicillin, potency was retained during 13 d. of storage, as evidenced by practically no acid production in these products when cultured and incubated at 95° F. for 8 hr.

**Condensed skim milk:** At the end of 10 d. of refrigerated storage of the product containing penicillin, acid production, in a portion inoculated at the rate of 3% of lactic culture and incubated at 95° F. for 7 hr., was insignificant.

**Non-fat dry-milk-solids:** Penicillin was added to skim milk at the rate of 1.0 unit/ml. It was dried in a table model experimental spray drier following the drying of a control sample of skim milk. Periodic examinations, after reconstitution to skim milk, revealed penicillin potency sufficient to greatly inhibit acid production of cultured portions, even when mixed 50-50 with some of the control. There was no detectable loss in potency of penicillin during a storage period of 10 wk. at room temperature.

**M31. Organic chelating agents as an aid to dairy detergency.** G. A. CLAYBAUGH and J. M. JENSEN, Michigan Agr. Expt. Station

Ethylene diamine tetra sodium acetate, used for the improvement of detergents, was studied in the laboratory by an experimental simulated washing test in a mechanical washing apparatus described previously by Jensen (J. Dairy Sci., 29: 453-463). The method measured the cleaning efficiency of the detergents in the removal of a prepared raw milk film from glass panes by selected washing procedures. These determinations, obtained by photometric readings, were made by measuring the percentage of light trans-

mitted through the washed panes. Commercially-prepared detergents, as well as experimental mixtures containing various amounts of the chelating agent were tested.

The chelating agent, when used in combination with condensed phosphates and wetting agents, was very effective in aiding the removal of all types of milk films studied. Such combinations easily removed raw milk films prepared by: (a) air drying, (b) oven drying followed by immersion in an alkaline detergent solution and (c) air or oven drying followed by immersion in a chlorine solution. Other combinations of the laboratory-prepared mixtures or commercial detergents such as organic acids, detergent sanitizers and alkaline detergents gave results indicative of incomplete cleaning. When either acid or alkaline detergents were used in hard waters which had been softened by the addition of the chelating agent, excellent detergency resulted. Even with the addition of 10% raw milk to the washing solution, chelating agent-detergent mixtures gave excellent results.

Many combinations of the chelating agent, condensed phosphates and wetting agents were found to be satisfactory detergents for the air-dried, raw-milk films. However, only a few combinations gave good results on the oven-dried, alkaline-detergent film, and the air-dried or oven-dried, chlorine film; these two films were the hardest to remove.

Preliminary data show that combinations of 20-80% chelating agent with a wetting agent and/or condensed phosphates give the highest detergency values. Actual practical applications using these laboratory-prepared mixtures, involving mechanical can washing, substantiate the laboratory detergency values for these compounds.

**M32. A comparison of phosphatase tests using different buffers, precipitants and periods of incubation.** GEORGE P. SANDERS and JOHN A. HUPFER, JR., Bureau of Dairy Industry, U.S.D.A.

The buffers studied included (a) barium borate-hydroxide, this Bureau's method; (b) sodium carbonate-bicarbonate, 21.65 g./l., pH 9.8, Cornell method; and (c) sodium carbonate-bicarbonate, 12 g./l., pH 10.05. Sensitivity was determined in terms of phenol, using 0.1% substrate and using BQC.

With milk and cheese, the sensitivity in the 1-hr. test was greatest with buffer *a* and least with *b*; in the 18-hr. test it was greatest on milk with *a* and on cheese with *c*. With buffer *c*, cloudiness in the color-development stage was eliminated by using potassium oxalate in the trichloroacetic acid precipitant and sodium metabo-

rate color development buffer; optimum pH for color development was found to be 9.3-9.4.

In the 1-hr. test, with buffers *a* and *c*, the optimum pH was about 10.0; with *b* the optimum pH curve was nearly flat with only a slight peak at 9.75. Buffer *c* yielded pH 10 with milk and 9.5 to 9.9 with cheese. It buffers adequately as a single buffer for various dairy products.

Sensitivity differences with these buffers may be due to inhibition by  $\text{HCO}_3^-$  and  $\text{CO}_3^{=}$  ions and/or ionic strength, decreased optimum pH with increased buffer concentration and long incubation, non-optimum substrate concentration in the 18-hr. test and unduly high pH for color development with buffer *b*.

**M33. Phosphatase measurements on high temperature vacuum pasteurized churning cream and ice cream mix.** G. H. WILSTER AND JUNIUS COVINGTON, Oregon State College

Samples were obtained at the discharge of the 3rd, or high-vacuum, chamber of the vacreator with the normal amount of product being continuously treated. By adjustment of the flow of steam used for heating, the temperature of the cream or ice cream mix in the low-vacuum pasteurizing chamber was varied from 200 or 205 to 170° F. with variations of 5 degrees. Samples were collected first when the highest temperature was used; the temperature then was decreased 5° at a time, allowing 3 min. between each temperature change before obtaining a sample. Fourteen lots of cream and 8 lots of ice cream mix were treated.

To determine if there was any reactivation of phosphatase or if there was any bacterial activity which would result in an increase in phosphatase, all of the samples of vacreated cream, as well as the buttermilk, were tested again at 24-, 48- and 72-hr. intervals. The finished butter was tested again after 7 and 14 d. of storage. All samples were maintained at 40° F. during the holding periods. The "Sanders-Sager" method was used. Negative results were obtained on all samples of heat-treated cream, ice cream mix and butter. Positive results were obtained on the raw products and on the buttermilk. There was no increase in phosphatase after storage for 24, 48 and 72 hr. in any of the samples of vacreated cream, ice cream mix or buttermilk. The butter did not show any increase in phosphatase after storage for 7 and 14 d.

**M34. Factors affecting production of proteolytic and coagulating enzyme by *Streptococcus liquefaciens*.** A. T. DUDANI AND F. E. NELSON, Iowa Agr. Expt. Station

The data on heat-inactivation, salting-out and optimum pH indicate that the proteolytic and coagulating enzyme activities of *Streptococcus liquefaciens* probably are just 2 manifestations of the same enzyme. The enzyme is adaptive in character, as all the 9 strains studied failed to produce any detectable amount of the enzyme when grown on Niven and Sherman's simplified amino acid medium (J. Bact., 47: 335-341. 1944). Two of the strains failed to grow on this medium without added thiamine (5  $\gamma$ /ml.). In a medium containing 2% vitamin test casein, 6 vitamins (as used in the Niven and Sherman medium), 1%  $\text{KH}_2\text{PO}_4$  and 0.2% lactose, the various strains produced different amounts of the enzyme as determined by the milk coagulation test and the freeing of tyrosine and tryptophan from casein.

In case of only one of the nine strains, addition of  $\text{B}_{12}$  (0.0004  $\gamma$ /ml.) to the casein medium resulted in a 3-fold increase in enzyme production. Substitution of  $\text{B}_{12}$  with cobalt failed to produce the same effect.

In milk, two of the strains showed maximum enzyme activity after 18 hr. incubation at 35° C. Incubation of cultures for longer periods resulted in gradual decrease and ultimate inactivation of the enzyme. This is believed to be due to longer exposure to the low pH of old milk cultures.

**M35. Taking representative milk samples from weigh tanks.** J. C. MARQUARDT, N. Y. State Dept. Agr. and Markets, Albany

An attempt has been made to identify the characteristics of weigh tanks which offer the best possibilities for representative sampling of milk for fat determinations. In a satisfactory tank, results on successive samples taken from the regular sampling port both immediately after dumping the milk and after vigorous agitation show no distinct trend to vary beyond the normal inherent variation of results by the Babcock method.

After correlating the capacity and the dimensions of 360 tanks with fat tests on milk from many of them, limited predictions are believed possible concerning the suitability of certain installations. Guided by this predictability, observations were made on 10 standard and on 5 special tanks. Determinations showed slightly better agreement in special tanks than in standard tanks. Establishing specifications for standard tanks may be unnecessary. New installations should be checked and old ones should be rechecked at regular intervals. Only experienced personnel should make conformance determinations.

A dye procedure has been used for determining

conformance characteristics in plants where objective checking as described above is difficult.

**M36. Determination of quaternary ammonium compounds in milk, and in detergent sanitizer and buffered quaternary solutions.** D. D. MILLER AND P. R. ELLIKER, Oregon Agr. Expt. Station

A modified eosin-indicator procedure has been developed for determination of concentration of added quaternary ammonium compounds (QAC) in milk and certain other foods. The method also has been adapted to determination of QAC in detergent sanitizer and in buffered QAC solutions. The essential steps in the procedure include: (a) Extraction and precipitation of QAC in a tetrachloroethane-acetone-eosin indicator solution; (b) Removal of interfering factors by washing the extraction solvent with successive portions of distilled water; (c) Titration of QAC extracted with standard anionic solution.

Recovery in the solvent fraction consistently has approximated 80% of the original QAC present in the sample. The remaining 20% can be recovered by repeating the extractions on the distilled wash water fractions. Trials included addition of 11 different QAC- and 3 QAC-containing detergent sanitizers to milk and tests to recover added QAC. Total recoveries within plus or minus 2 ppm. were obtained on concentrations ranging from 5–100 ppm. QAC in milk. Some QAC preparations could be detected in milk in concentrations lower than 5 ppm.

**M37. Ion exchange as a means of improving the keeping quality of frozen homogenized milk.** H. S. HALLER AND R. W. BELL, Bureau of Dairy Industry, U.S.D.A.

Ion exchange treatment of milk, in most instances, deferred the formation of an oxidized flavor during frozen storage. However, some exchangers imparted a slight salty or other foreign flavor when the treatment was too vigorous or prolonged. The physical stability of the frozen milk varied with different types of treatment. Removal of about a 4th of the Ca and lesser amounts of the other cations with no removal of the citrates, phosphates or chlorides resulted in definite improvement in physical stability. However, removal of approximately these same amounts of cations together with half of the citrates, phosphates and chlorides greatly reduced the stability. Removal of about 20% of the citrates and a small amount of Ca and other cations resulted in a slight decrease in stability.

The most satisfactory treatment involved the use of an organic cation exchanger regenerated to a 99:1 sodium-hydrogen ratio. This treat-

ment, by removal of as little as 10–15% of the Ca and lesser amounts of the other cations, with no removal of the anions, produced a normal flavored milk with improved physical stability and deferment of an oxidized flavor.

**M38. Observations on the effect of additions of heat thickened protein to fluid milk on the creaming phenomenon.** A. C. SMITH AND F. J. DOAN, Pennsylvania State College

Some eastern milk dealers have been increasing the cream volume of their bottled milk by the illegal practice of standardizing high test milk by addition of reconstituted superheated condensed skim milk. The expansion of the cream volume apparently depends on the entanglement of protein flocs in the rising fat globule aggregates and its degree is influenced by the amount of superheating of the adulterant and by the proportion added. Maximum increases in cream volume result when the ratio of fat to superheated protein ranges between 2.85 and 4.0.

Positive detection of this type of adulteration is not easy. Adulterated milk exhibits normal appearance, flavor, alcohol number, pH, freezing point, surface tension and tyrosine values. The nitroprusside test for —SH groups, the Evanson test for "bound" lactose and the test for furfural on the steam distillates are negative. Microscopic observation (with and without staining techniques) of the mixed milk and of the cream layers reveals no distinguishing criteria. The curd content of butter churned from the cream layers is normal.

Adulterated milk shows more sediment on centrifuging and a higher ratio of casein to albumin and globulin; the cream layer exhibits sub-normal fat-to-casein and fat-to-s-n-f. ratios; and the under layer is higher than normal in viscosity by the Ostwald pipette. The fat-to-s-n-f. ratio of the cream layer appears to be the most convenient of the significant criteria for detection of the adulteration.

**M39. The effectiveness of some antifoaming agents in the condensing of skimmed milk and whey.** J. ROBERT BRUNNER, Michigan Agr. Expt. Station

Milk, cream and butter as well as selected surface active compounds were added to skimmed milk and whey to control excessive foaming during the condensing operation. Fresh cream and sweet cream butter, used to provide a milk fat concentration not exceeding 2% in the skimmed milk and whey, were moderately effective as foam depressants. However, the addition of much smaller amounts of slightly rancid milk or cream were extremely effective.

Surface active compounds used were divided into 2 groups according to their respective solubility in water. The water-insoluble materials, listed in the order of their effectiveness as anti-foaming agents, were silicone materials, mono- and diglycerol esters of high molecular weight fatty acids, sorbitan monolaurate and octyl alcohol. The water-soluble, surface-active materials studied were butyric acid, a cationic quaternary ammonium chloride, an anionic alkyl aryl sulfonate and a nonionic polyoxyethylene sorbitan monolaurate. The foam-inhibiting property of butyric acid and the cationic quaternary was pronounced, but in both instances the collapse of the foam film was accompanied by a partial coagulation of the milk proteins. The addition of either polyoxyethylene sorbitan monolaurate or the alkyl aryl sulfonate to skimmed milk and whey enhanced the formation of copious amounts of a very stable foam.

Since the water-insoluble, surface-active compounds were most effective as antifoaming agents during the condensing of skimmed milk and whey, the foam depressing property of rancid milk and cream possibly could be attributed to the presence of water-insoluble, surface-active materials formed as a result of the partial hydrolysis of milk fat. With the exception of octyl alcohol, none of the antifoamants, when used in effective concentrations, imparted an off-flavor to the condensed products.

**M40. The antioxidant properties of nordihydroguaiaretic acid in cream pasteurized at various temperatures.** B. T. KARNANI, DIONISIOS A. THEOKAS AND VLADIMIR N. KRUKOVSKY, Cornell Univ.

The addition of NDGA antioxidant to portions of cream prior to their pasteurization at 143, 150, 160 and 170° F. for 30 min. (0.005% of the bulk fat) and which were either depleted of the total vitamin C content or to which vitamin C (20 mg./l.) and Cu (0.4 mg./l.) were added alone or together, resulted not only in the increase in apparent tocopherol values of the fat, but also in the stabilization of cream and fat against deterioration (as determined by the re-emulsification test) for at least 180 d. at sub-zero temperatures and for an additional 20 d. at 0-1° C.

The pasteurization of cream at 143 and 150° F. without NDGA antioxidant resulted in the prevention of oxidized flavors in both cream depleted of the vitamin C content and cream containing added vitamin C for 180 d. at subzero temperatures and an additional 20 d. at 0-1° C. However, the fat became unstable at the end of 75 d. at sub-zero temperatures, and, in the case of creams pasteurized at 143° F., at the end of 10

and 20 d. at 0-1° C. after approximately 60 d. at sub-zero temperatures. The creams containing Cu or added Cu and vitamin C developed oxidized flavors at the end of 75 d. at sub-zero temperatures, and the fat became unstable at the end of 15 d. at sub-zero temperatures.

The pasteurization of cream at 160 and 170° F. without NDGA antioxidant prevented the oxidized flavors in cream depleted of the vitamin C content, and cream containing added vitamin C for 180 d. at sub-zero temperatures and the additional 20 d. at 0-1° C. During this time only the fat from cream pasteurized at 160° F. and containing added vitamin C became unstable at the end of 75 d. at sub-zero temperatures. The same cream, but containing Cu or Cu and vitamin C developed oxidized flavors at the end of 45 d. at sub-zero temperatures, and the fat became unstable at the end of 15 d. at sub-zero temperatures. The creams pasteurized at 170° F. and containing Cu alone and together with vitamin C developed oxidized flavors, and the fat became unstable at the end of approximately 10 d. at 0-1° C. following 180 d. at sub-zero temperatures.

**M41. Stability of evaporated milk as influenced by various conditions of homogenization.** R. B. MAXCY AND H. H. SOMMER, Univ. of Wisconsin

Applying the normal process of manufacturing evaporated milk, the temperature of homogenization had little, if any, effect on the heat stability of the finished product. On the other hand, if the milk had been homogenized prior to forewarming and condensing, the temperature of homogenization exerted a profound influence on the final stability. There was a marked increase in stability as the temperature of homogenization was increased from 100° F. up to near 180° F.

When the milk had been homogenized at 160° F. or above as unconcentrated milk, the stability could be improved 5-6 min. at 242° F. by re-homogenizing the product at 130° F. after it had been concentrated.

When cream containing 20% butterfat was homogenized at 136° F., then mixed with forewarmed and concentrated skim milk to obtain a product with the composition of evaporated milk, the product was fairly stable, but the stability could be improved 5-6 min. at 242° F. by re-homogenization at 130° F.

Evaporated milk prepared in the normal manner was homogenized as many as 7 times at 130-135° F. without detrimental effect to the stability beyond that of the first homogenization.

**M42. Separation of fat and protein in sterilized milks during storage.** B. H. WEBB, E. F. DEYSHER, C. F. HUFNAGEL AND F. E. POTTER, Bureau of Dairy Industry, U.S.D.A.



Sterilized milks of different concentrations, including evaporated milk, exhibit various degrees of fat and protein separation during storage. Excessive separation is objectionable and it does not occur in properly processed evaporated milks held under normal storage conditions. Poor homogenization, inadequate heating during sterilization, low viscosity and high storage temperatures accelerate phase separation. Thus far, it has not been possible to improve the color and flavor of these milks by rapid sterilization because of the destabilizing influence of this kind of sterilization on the fat and protein dispersion. Two high-temperature short-time methods may be used to sterilize plain or evaporated milk: (a) sterilize the milk in cans with violent agitation at about 260° F. for 3 min. and (b) sterilize the milk with steam jets or in a tubular heater at about 285° F. for 15 sec., then package it aseptically. Milks so sterilized have a very thin body, which allows rapid separation of fat and protein, and a storage life of only a few months. When protein separation in sterilized milk does occur, it appears first as a precipitate, but in later stages, the high localized concentration of protein seems to be conducive to gel formation. If the protein settles, the gel appears on the bottom of the can. If the protein is adsorbed on the fat phase, which rises, redispersion of the gel-like fat layer becomes almost impossible. Phase separation proceeds more rapidly in sterilized skim milk than in whole milk. The fat and protein, when bound together by adsorption, partially stabilize each other. If all of the fat and protein of milk could be combined by adsorption and dispersed as small units, a balance might be obtained which would retard separation regardless of the viscosity of the milk.

**M43. The use of concentrated essence for improving the flavor of strawberry and peach ice cream.** C. C. FLORA, L. L. DAVIS AND C. W. HOLDAWAY, Virginia Agr. Expt. Station

In the Horticultural Dept. of the Virginia Agr. Expt. Station, concentrated strawberry and peach essences were processed by a specially designed fruit-essence recovery process for supplying concentrated volatile flavors. These essences were used with frozen and canned strawberries and peaches in making strawberry and peach ice cream.

When concentrated strawberry and peach essences prepared by the above process were added to fruits for use as an ice cream flavor, they fortified and greatly improved the natural flavor of this fruit. This fortified flavor carried through to the finished ice cream. The improvement in flavor seemed to be somewhat better when essences

were used with the canned strawberries and peaches rather than with the frozen strawberries and peaches. Concentration of 20 ml. of approximately 100-fold essence/5 gal. of mix rated superior to that of higher or lower concentration.

**M44. Some relationships of the oxidation-reduction systems of milk to the keeping quality of the dry product.** H. A. HARLAND, S. T. COULTER AND R. JENNESS, Univ. of Minnesota

Twenty lots of dry whole milk were prepared from fresh milks that had been subjected to a variety of preheating conditions ranging from 85° C. for 25 sec. to 96° C. for 30 min. These milks were examined following each step in the processing for their oxidation-reduction potentials and assayed for substances that reduce thiamin disulfide, acid ferricyanide, O-iodosobenzoate and 2,6-dichlorophenolindophenol. In addition to these tests, flavor scores and fat peroxides were determined on the dry milks initially and following biweekly intervals of storage in air at 37° C.

Treatment of the fluid milk at 96° C. for 75 sec. produced dry whole milk with the best keeping quality as judged organoleptically and by means of fat peroxide determinations on the extracted fat. Moreover, this heat treatment produced the maximum amount of thiamin disulfide-reducing substances (TDRS) in the milk. While there may be no direct proof for the anti-oxigenic properties of TDRS in milk, a close relationship exists between the amount of TDRS present in dry whole milk and the storage life of this product in air.

The iodosobenzoate-reducing substances in milk decrease with increasing time of heat treatment of fluid milk in air but do not show further decrease during 10-wk. storage of dry whole milk in air at 37° C. Furthermore, no direct relationship is evident between the quantity of iodosobenzoate-reducing substances and fat deterioration during storage of dry whole milk.

Since the oxidation-reduction potential of milk depends largely upon the O<sub>2</sub> tension and the ascorbic acid content, determinations of the relatively small changes in E<sub>h</sub> that occur during processing and storage of dry whole milk are of limited value for studies of the relationships of processing changes in milk to the keeping quality of the dry product.

**M45. A disc method of filtration for roller process non-fat dry milk solids.** D. R. STROBEL AND C. J. BABCOCK, U. S. D. A.

The filtration method for determining sediment content generally is more accurate, more uniform and faster than the tumbler method. However, the insoluble solids in reconstituted roller nonfat

dry milk solids prevent sediment determination by filtration through the standard lintine disc. A filtrable solution was produced by a pepsin-hydrochloric acid solution (10 g. pepsin in 500 ml. water plus 25 ml. HCl, diluted to 1,000 ml.) prepared as follows: Mix 25 g. of the milk powder in 125 ml. of the solution, heat to 40° C., hold 40 min., boil 10 min., then filter. The sediment content determined by this method equaled or exceeded that determined by the tumbler method and gave a truer and more complete picture of the sediment content of the samples than that shown by the tumbler method. The discs clearly showed scorched particles, burnt particles and other sediment.

Results will be presented of tests on 200-300 samples, comparing the best established procedure with the tumbler method.

**M46. Comparison of the yield of non-fat dry milk solids when using three types of spray drying equipment.** BEN M. ZAKARIASEN AND GUNNAR E. NELSON, Land O'Lakes Creameries, Inc., Minneapolis, Minn.

In recent months many inquiries have been received as to which type of spray drying equipment will produce the best product at the lowest cost per pound. An important factor in determining which type of drying equipment to purchase, is the yield of non-fat dry milk solids per pound of solids in the original liquid skim milk.

In this study, an attempt was made in a practical way to compare the yield using 3 types of commercial spray drying equipment. One type was inferior from the standpoint of yield of non-fat dry milk solids per pound of solids in the original skim milk.

**M47. Effect of heating on the diffusion of calcium, magnesium, phosphorous and citric acid.** T. D. HARMAN AND W. L. SLATTER, Ohio State Univ.

The effect of heating skim milk on the rate of diffusion of Ca, Mg, P and citric acid was studied using an adaptation of the Hanke and Koessler method of dialysis. Fresh skim milk was dialyzed against an isotonic solution of lactose and NaCl, using a cellophane membrane calibrated with a standard solution of these ions. Two series of heat treatment were used. In the 1st, skim milk was heated to 62, 77 and 100° C. for 30 min., and in the 2nd series, the skim milk was heated to 100° F. for 30, 60 and 90 min. In each case raw milk served as a control.

In all cases the rate of diffusion of the Ca, P and Mg was decreased markedly, whereas the citric acid apparently was unaffected. The decrease in the rates of diffusion of the Ca, Mg and

P was essentially the same when the skim milk was heated to the various temperatures for 30 min. The extension of the heating period to 60 and 90 min. resulted in further decreases in the rates of diffusion which were not directly proportionate to the increased time of heat treatment.

In the case of Ca, Mg and citric acid, the diffusion resembled a first order reaction, but the P diffusion did not.

**M48. The operation of a spray drier at high temperature and under pressure.** V. H. TOWNLEY AND S. T. COULTER, Univ. of Minnesota

A pilot model of the Minnesota spray drier, equipped with a gas burner to supplement the steam heaters, was used to study the effects of varying the inlet air temperature and the absolute air pressure on (a) the drying rates, (b) the temperature of the powder during drying, (c) the moisture content of the powder produced and (d) the solubility index of the powder.

Both high-temperature inlet air and high absolute air pressure in the drier would increase the capacity and the former would increase the thermal efficiency of the drier. The time necessary to dry the powder to 5% moisture content apparently was dependent on the nozzle characteristics and on the water vapor pressure of the exit air. Neither the inlet air temperature nor the air pressure had any apparent effect on the temperature of the drying particles nor upon the solubility index of the powder. To maintain a constant outlet air temperature, it was necessary to increase the milk flow 300% with increase in inlet air temperature from 270 to 410° F. Increase in air pressure necessitated an increase in milk flow in direct proportion to the density of the air in order to maintain constant outlet air temperature. The use of these high milk flow rates caused an increase of 1.5% in the moisture content of the powder, presumably due to increase in a vapor pressure of the outlet air. This increase in moisture content would not be a bar to the use of these methods since a redrier could be used to remove the excess moisture.

**M49. The changes produced in the ultrafilterable calcium, phosphorus, and nitrogen components of skim milk during processing in a Mallory heat exchanger.** E. A. BERNARDONI AND S. L. TUCKEY, Univ of Illinois

Six lots of fresh skim milk, one lot of skim milk plus 0.06%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , and 2 lots of sweet whey each were heated in a Mallorizer to the following temperatures: 325, 300, 250 and 200° F. The samples were filtered through a no. 2F porcelain Pasteur-Chamberlain ultrafilter in a Briggs chamber under 90 lb. pressure.

The ultrafiltrate from each lot was analyzed for: total N, amino N, Ca and P. The average total N content of the filtrate from the skim milk samples heated to 200, 250, 300 and 325° F. was 92.4, 87.4, 93.6 and 104.6%, respectively, of the raw skim milk filtrate. However, the whey samples showed a continuous decrease in total N with increased temperatures. The amino N content in the filtrate from the skim milk samples increased as the temperature was raised above 250° F. The Ca and P content in the ultrafiltrates decreased as the temperature increased. At 325° F., the average decrease was 15.7 and 10.4%, respectively, when compared with the filtrate from the raw sample. At 325° F. for the whey sample this decrease amounted to 30.3% for the Ca and 13.7% for the P.

**M50. Effect of high temperature short time heat treatments of milk on the denaturation of albumin and globulin.** J. H. HETRICK, Dean Milk Co., Rockford, Ill. AND P. H. TRACY, Univ. of Illinois

Milk was heated, held and cooled continuously by means of a Mallory heat exchange unit. Calculated times of 4.2 and 1.6 sec., respectively, were required to heat the milk to and cool the milk from the desired holding temperatures.

Total N, non-casein N and non-heat denaturable N were determined in duplicate on the experimental samples. The % of the total amounts of albumin and globulin denatured were calculated both from the increases in casein N and from the decreases in albumin and globulin N. No attempt was made to determine either albumin N or globulin N separately so that the data refer to the denaturation of a fraction which includes both albumin and globulin. The percent of the total albumin and globulin denatured by a standard heat treatment was reproduced satisfactorily by the methods employed when different lots of milk were used. These values were not affected by variation in the total N content.

When the heat treatment employed was limited to minimum time-temperature conditions for inactivation of the phosphatase, no denaturation of albumin and globulin occurred. The extent of denaturation of albumin and globulin with time at a constant temperature of 170° F. followed closely the first order law, but at 230° F. this was not the case. Heat treatments sufficient to cause the first noticeable cooked flavor resulted in the denaturation of about 58% of the albumin and globulin. Data are given showing amounts of albumin and globulin denatured by heat treatment in the temperature range from 180 to 322° F. with calculated holding times at top tempera-

ture of 0.03, 26 and 64 sec. Data also are given which show the importance of rate of heating to a given temperature on the amounts of albumin and globulin denatured.

**M51. Lactose degradation in heated milk.** STUART PATTON, Pennsylvania State College

Earlier work demonstrated that furfuryl alcohol, maltol and hydroxymethylfurfural are formed from lactose in heated milk. Present studies are concerned with the conditions which favor the generation of these compounds. Condensed skim milk (30% total solids) and a number of simplified systems, employing lactose in combination with other materials, were used in these experiments. The compounds were isolated from the heated samples by a continuous ether extraction technic.

Milk samples heated 127° C. for 2.5 hr. at pH 6.5, formed very little hydroxymethylfurfural but significant quantities of maltol and furfuryl alcohol. The reverse was observed to be true with milk samples adjusted to pH 4.8 prior to heating. Results with the heated lactose systems indicated that weakly basic conditions favor the production of furfuryl alcohol. This compound was isolated from systems of lactose combined with casein, lysine, or  $\text{NaHCO}_3$ , and in smaller amounts from a heated solution of lactose in phosphate buffer (pH 6.6). In conjunction with the experiments on furfuryl alcohol, it was observed that amino acids or casein are somewhat specifically required for the heat generation of maltol from lactose. Maltol was obtained from heated systems of lactose combined with casein, lysine and, to a lesser extent, glycine. Heating lactose in the presence of dilute  $\text{NaHCO}_3$ ,  $\text{NH}_4\text{OH}$  or  $\text{NaOH}$  produced no maltol.

The heat degradations of glucose and galactose were studied under a wide variety of conditions but in no instance was furfuryl alcohol or maltol observed to be formed from these sugars.

## EXTENSION SECTION

**E1. Master package for integrated county meetings on dairy farm management.** STANLEY N. GAUNT, Massachusetts State College

Our Extension Dairy Committee each year reviews the problems of the industry and develops an educational program to meet them. To meet this year's major problem of adjusting farm operations to smaller net incomes because of lower milk prices and high production costs, a "master package for integrated county meetings on dairy farm management" was developed. This package consisted of 4 meetings with the first one presenting the situation from a survey of production costs and how dairymen can analyze their

problems. The 3 following meetings covered how to increase the net farm income.

Development of this package included: (a) the organization of subject matter and its assignment to 7 specialists; (b) preparation of supporting and supplementing subject matter; (c) planning and constructing effective visual aids; (d) a preview before county agents of each specialist's talk; and (e) preparation of suggested circular letters, news stories and highspots of each meeting.

The preview convinced county agents that these packaged meetings were different and better. The result was more dairy meetings, particularly in certain counties, more dairymen attending and, according to most people, the best organized and presented series of dairy meetings held in Massachusetts in recent years.

**E6. Effective methods in promoting the junior dairy project.** GLEN W. VERGERONT, Univ. of Wisconsin

Extension dairymen working in an advisory capacity with state 4-H Club departments and county extension agents have suggested several plans which have been helpful in promoting the junior dairy project. State breed associations, service clubs and active groups of dairymen have helped boys and girls to locate, finance, select and draw suitable calves on various practical plans. Judging schools, judging contests, tours, fitting and showing demonstrations, heifer sales, dairy demonstrations and twilight meetings have helped advance the work.

Suggested quarterly and yearly plans of work are used in many states to get an orderly approach to the teaching of efficient dairy production fundamentals as the activities enumerated above.

District project meetings for county extension agents and leaders and some county leadership meetings have been assisted by extension specialists.

Visual aids have been used at meetings and handicraft projects and practices have been suggested in the order of their popularity as follows: rope halters, blankets, hay racks, techniques in foot trimming, feed boxes, calf stalls, false bottom calf pens, tools, electric dehorning and milk stools.

**E7. How the American Dairy Science Association and the United States Department of Agriculture can aid in the development of 4-H Dairy Club plans and programs.** E. W. ARRON, U.S. D.A., Washington

In Dairy 4-H Club work there can be a happy combination of emphasis which results in a 4-

fold development of the boy or girl and the economic progress of the farm and community. Dairy members remain in Club work longer than those who enroll in most other projects. Dairy and livestock projects are well adapted to the development of family partnerships with the parents. 4-H education in meal planning helps build strong bodies and the kind of teeth that encourage youth to smile and enjoy life.

The American Dairy Science Association and the Cooperative Extension Services of the United States Department of Agriculture and the States should develop some joint projects of research and study. A few problems or questions that might be tackled for a start are:

1. What are the "success factors" of our present 4-H Dairy programs?

2. Is it educationally sound to expect youth of 10 and young adults of 20 to participate on the same basis in the same program and the same club?

3. Is individual competition of one member vs. other members the best and only effective stimulation toward greater effort? Is it possible that some of the group competition used so successfully in the Young Farmers Clubs of England, Scotland, Wales and Ulster might also prove to be successful here in the United States for holding the interest of older members?

4. Many new educational methods and techniques have been devised in the past 30 yr. Have we kept pace?

5. What are the values and methods of bringing more youth participation into the planning and action phases of 4-H Dairy programs?

**E11. Hyperkeratosis (X-disease) of cattle.** P. OLAFSON, Cornell Univ.

Hyperkeratosis is a chronic disease of cattle of unknown cause. Over 30 scattered outbreaks of the disease have been diagnosed in New York State since the original recognition of the condition in 1941. The disease has appeared in more than 30 states.

This is an insidious chronic disease which may have been in progress for weeks before the owner suddenly realizes that serious trouble is present. The early manifestations are interpreted as lousiness, mange or other skin diseases. Antiparasitic treatments fail and the disease continues to progress. The chief symptoms are watery discharge from the eyes, salivation, loss of weight, depression and a progressive thickening and wrinkling of the skin. The skin changes start over the withers, sides of the necks and on the cheeks. Severe cases may lose most of the hair and the upper two-thirds of the body may be covered by altered skin. Wart-like proliferations

may be present in the mouth. Examination of the internal organs will reveal marked changes in the liver, kidneys and pancreas. There is proliferation of the bile and pancreatic ducts, as well as cystic dilatation of kidney tubules. Affected animals have little resistance to pus-producing bacteria which results in frequent development of mouth, skin and liver abscesses. Young animals ranging from 4 to 24 mo. of age are affected most frequently.

Experiments are being conducted to determine whether this is an infectious, toxic or metabolic disease. The problem has proven to be a difficult one and so far no one has succeeded in producing the disease experimentally.

**E12. Method of conducting an educational program for area brucellosis control.** E. C. SCHEIDENHELM, Rutgers University

The New Jersey dairy extension program has a sub-project entitled, "Farm sanitation and animal health". This, in the main, is an educational program complementing the regulatory program of the State Bureau of Animal Industry. Through a long-time program an attempt is being made to have the entire state become a modified accredited area for brucellosis.

Area work for control of brucellosis has been set up on a township test basis. Counties with low cow population are used to start with and eventually all counties will be included. Steps in developing a township program are: (a) Convince agricultural agent program is necessary. (b) Meet with county board of agriculture and secure their approval. (c) Educational meeting to get a majority vote of farmers in the selected township. All testing is done on a voluntary

basis. Each dairyman uses results to select the brucellosis control program best adapted to his particular problem.

**E14. Bacteria in the cow's udder associated with mastitis.** J. J. REM, Pennsylvania State College

An investigation of mastitis has been conducted during the past 9 yr. It has included studies of the organisms associated with udder trouble and the relation of certain management practices to the incidence of the disease. More than 40 herds have been included in the investigation representing institutional and farm herds throughout Pennsylvania. The relative incidence and importance of *Streptococcus agalactiae* has been overemphasized in the literature. Data obtained indicate that this organism is seldom, if indeed ever, the agent found associated with an initial case of mastitis but rather the organism which may eventually become of importance following a period in which udder resistance falls to an extremely low level.

Organisms found associated with initial cases of udder trouble include pyogenic *Micrococcus* species, coliforms and, particularly in the case of older animals, *Streptococcus* species other than *agalactiae*. *Pseudomonas aeruginosa* has been found with some of these organisms in certain herds. *Corynebacterium* species do not appear to be of importance in mastitis developing during lactation. In many cases it has been found impossible to isolate bacteria from milk obtained at the onset of trouble.

Data further indicate that the cause of mastitis is related to management practices or injuries which lower the resistance of the udder. The organism eventually found associated with the trouble is purely adventitious in nature.

# DETERMINATION OF MILK MINERALS BY FLAME PHOTOMETRY<sup>1</sup>

R. J. KEIRS AND S. J. SPECK<sup>2</sup>

*Department of Dairy Science, University of Illinois, Urbana*

This article is an outgrowth of the authors' need for a method for the determination of the minerals in milk during a milk composition study involving the determination of some 15 to 20 samples per month over a 3-yr. period. The authors sought a method not as laborious nor as time consuming as the standard chemical methods but still having an accuracy within 1 to 2 per cent of the total amount present. The investigation was undertaken to establish a flame photometric method for calcium, sodium, potassium and magnesium using a Beckman D.U. spectrophotometer with flame attachment (17). The authors succeeded in the cases of the first three elements, but failed to establish a method for magnesium, principally because of the low concentration of magnesium in milk. Milk averages 0.012 per cent magnesium (10), while the upper limit of concentration of magnesium determinable to 1 per cent of the amount present by the Beckman flame photometer is 0.2 per cent (17).

Flame photometry is a relatively new adjunct to analytical chemistry. Several papers (2, 3, 4, 9, 16, 19, 20) have described flame photometric methods, techniques and improvements for samples including biological materials using instruments other than the Beckman. Brown *et al.* (6) describe a method for calcium and magnesium in leaves using a Beckman instrument, but no method has been published specifically for minerals in milk.

Several techniques were studied before deciding upon the method described. The absolute method of determining an unknown concentration by interpolating flame intensity readings on a preplotted working curve prepared from known standard solutions first was tried with little success regarding accuracy and reproducibility. The internal standard technique proposed by Gerlach (7) for spectrographic analysis and applied to flame photometry by Berry *et al.* (3) also was investigated. While this latter technique increased precision over the absolute method, results still were not satisfactory. A modification of the absolute method similar to that employed by Gilbert (8), but simpler in that background readings and a calcium background curve are eliminated, was studied and employed with success. In this method the concentration of the unknown is determined by interpolating the relative intensity of the unknown between the in-

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<sup>2</sup> Present address: Dairy Department, University of Georgia, Athens.

tensities of two standards, one of slightly lower concentration and the other of slightly higher concentration than the unknown. The three readings are taken within seconds of each other to reduce chance for variation in the flame or instrument during the readings.

#### EXPERIMENTAL

A Beckman D.U. spectrophotometer (17) with flame attachment was used throughout this study. Although numerical results were not tabulated, it was observed visually that with illuminating gas a steadier response was obtained on the galvanometer than when propane was used. For this reason, illuminating gas was used throughout. The gas pressure was maintained at 3.5 cm. of *n*-butyl alcohol (manometer fluid). This pressure gave a clear cone-shaped flame at oxygen pressures used for each of the elements without danger of back-firing. The optimum oxygen pressure was determined for each element by plotting the spectral intensity of each element as oxygen pressure was varied.

It was necessary to preheat the spray chamber at least 10 min. before taking the first of a series of readings to allow the chamber to reach an equilibrium temperature. The solvent used was atomized into the spray chamber during this preliminary heating; otherwise the temperature would rise above the operating temperature and the first few readings taken would be erratic until the equilibrium temperature was established again. Alcoholic solutions (20 per cent by volume) gave a steadier flame and were used in preference to aqueous solutions.

Correction for flame background was not made. Preliminary investigation showed that, for a given slit width and with the preparation of standards of the approximate composition as the unknowns, the flame background at any particular wavelength remained constant and was the same for a given ion whether in standard or unknown solution.

The effect of each of the ions in solution upon the intensities of calcium, potassium and sodium was studied. This was done by preparing, as a standard of reference, a solution with the following ion concentrations: calcium, 12 p.p.m.; potassium, 12 p.p.m.; sodium, 4 p.p.m.; phosphorus, 12 p.p.m.; magnesium, 1 p.p.m.; hydrochloric acid, 0.2 ml. 1 + 1 HCl per 100 ml.; and 20 ml. 95 per cent ethyl alcohol per 100 ml. This solution served as a standard in the determination of the effects of the various ions and HCl upon the apparent p.p.m. of calcium, potassium and sodium. Other groups of solutions were prepared having the same ion concentrations as this standard except for one element which varied in concentration, as for example, from 0 to 24 p.p.m. for calcium, 0 to 8 p.p.m. for sodium and 0 to 24 p.p.m. for potassium.

In the concentration ranges studied magnesium did not affect the intensity of calcium, potassium nor sodium. Phosphorous as phosphate likewise did not affect the determination of sodium or potassium, but did have a marked effect upon calcium (fig. 1). This effect is a function of concentration up to 7 p.p.m. phosphorus but becomes constant for phosphorus concentrations above 7 p.p.m.;

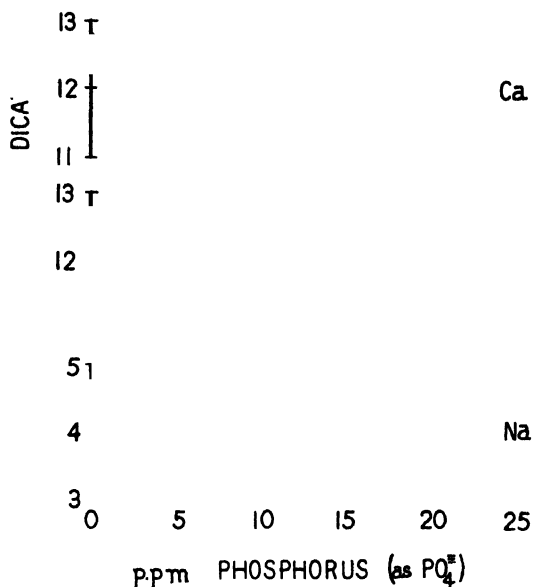


FIG. 1. Effect of phosphorus upon the apparent p.p.m. of the elements indicated.

hence the effect of phosphorus upon calcium was reduced to a negligible amount by incorporating phosphorus in all standard solutions in an amount greater than 7 p.p.m. Fig 2 shows a similar effect of HCl upon potassium. Here again,

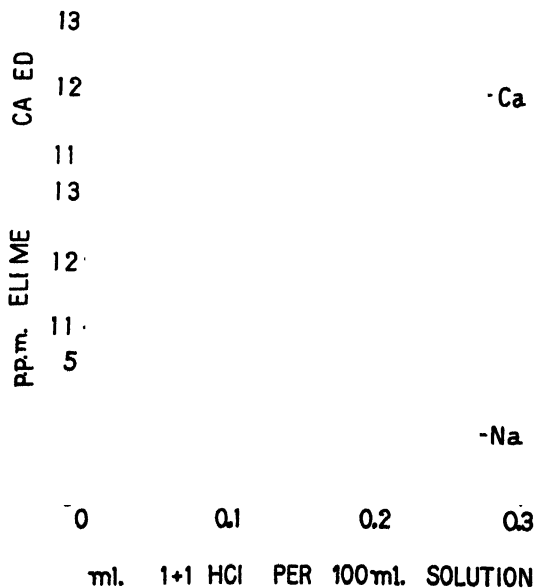


FIG. 2. Effect of HCl upon the apparent p.p.m. of the elements indicated.



the effect was minimized by maintaining the HCl concentration at or above 0.15 ml. 1 + 1 HCl per 100 ml. solution.

Although potassium and sodium have no effect upon the calcium intensity, they do have an effect upon each other. Fig. 3 indicates the effect of sodium

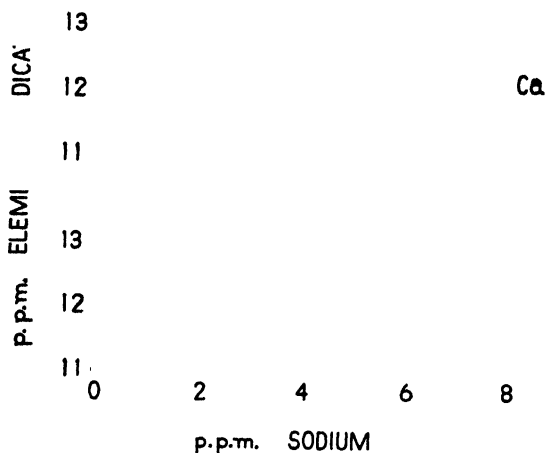


FIG. 3. Effect of sodium upon the apparent p.p.m. K and Ca.

upon potassium and fig. 4, the effect of potassium upon sodium. In both cases, the effect varies over the whole concentration range investigated, hence a correction must be applied in each case unless the unknown solution contains the same concentration of these two ions as the standards.

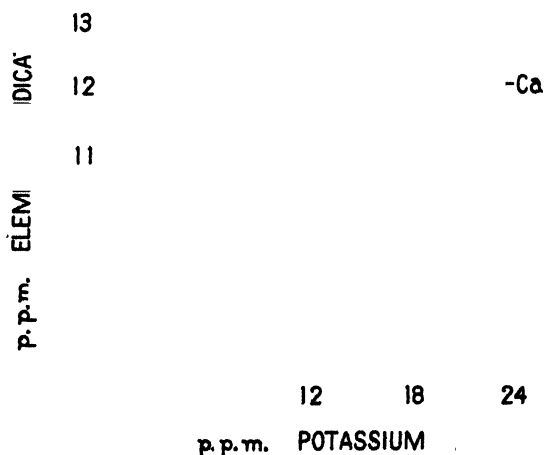


FIG. 4. Effect of potassium upon the apparent p.p.m. Ca and Na.

Sodium was added to each standard at a concentration of 4 p.p.m. Potassium was added at a 12 p.p.m. concentration. Hence, for each p.p.m. potassium difference from 12 p.p.m. in the unknown, a correction of 0.03 p.p.m. sodium was

made for the sodium concentration. This correction was added if the concentration of potassium were above 12 p.p.m. and subtracted if the concentration were below 12 p.p.m.

Similarly, a correction for potassium was necessary when the sodium concentration differed from 4 p.p.m. A correction of 0.13 p.p.m. potassium was made for each p.p.m. sodium difference from 4 p.p.m. The correction was added when the sodium concentration in the unknown was greater than 4 p.p.m. and subtracted when sodium was below 4 p.p.m.

Theoretically, a determination involving an intercorrection of this sort is not a good one. In routine milk analysis, however, where the sodium and potassium concentrations varied between relatively narrow limits, by establishing the sodium concentration in the potassium standards at 4 p.p.m. and the potassium concentration in the sodium standards at 12 p.p.m. (the approximate concentrations of these two elements in the diluted milk samples), the correction due to the effects of either of these two ions on the other was small, and, in the majority of cases, within the experimental error.

*Preparation of sample stock solutions.* The milk to be analyzed was warmed to approximately 21° C., mixed thoroughly by pouring from one container into another and a 100-g. sample weighed accurately into a 300-ml. porcelain crucible. One-half ml. glacial acetic acid was added to prevent the formation of surface film during drying and the sample dried at 100° C. for 24 hr. When dry, the sample was placed in a muffle furnace and the temperature gradually raised to 550° C. After cooling, the ash was dissolved by leaching for 30 min. with 10 ml. of 1:1 HCl and approximately 100 ml. of hot water. This solution then was filtered through ashless filter paper into a 500-ml. volumetric flask. The filter paper was returned to the porcelain crucible and the ashing, leaching and filtering were repeated to insure complete ashing and extraction. Following the second filtration, the filtered solution was made to 500 ml. with distilled water. Aliquots of this solution were used for analysis. This solution will be referred to as the sample stock solution.

*Preparation of standard stock solutions.* A sodium stock solution was prepared by dissolving reagent grade NaCl in distilled water to give a solution containing 4 mg. sodium per ml. A potassium stock solution was prepared by dissolving reagent grade KCl in water to give a solution containing 12 mg. potassium per ml. A calcium stock solution was prepared by dissolving pure CaCO<sub>3</sub> in dilute HCl, warming and diluting with water to give a concentration of 12 mg. calcium per ml.

In addition, a solution containing phosphate was prepared from syrupy phosphoric acid (85 per cent) to give an approximate concentration of phosphorus of 12 mg. per ml. The exact phosphorus concentration was determined chemically, using the ammonium molybdate method of Pemberton (21) and Kilgore (13) and modified by Hibbard (11).

*Preparation of solutions to be atomized into flame.* The unknown solutions atomized into the flame were prepared by pipetting 5-ml. aliquots of the sample stock solution into a 100-ml. volumetric flask, adding 20 ml. of 95 per cent ethyl

alcohol and diluting to the mark with distilled water. This solution was used for the determination of calcium, sodium and potassium.

Standard solutions atomized into the flame were prepared from aliquots of the standard stock solutions to give solutions with the following compositions:

*Calcium standards*

Calcium	8, 11, 14, 17 p.p.m.
Phosphorus	12 p.p.m.
Hydrochloric Acid (1 + 1)	0.2 ml. per 100 ml. solution
Ethyl Alcohol (95%)	20 ml. per 100 ml. solution

*Potassium standards*

Potassium	7, 10, 12.5, 15, 17 p.p.m.
Sodium	4 p.p.m.
Hydrochloric Acid (1 + 1)	0.2 ml. per 100 ml. solution
Ethyl Alcohol (95%)	20 ml. per 100 ml. solution

*Sodium standards*

Sodium	2, 4, 5, 6.5 p.p.m.
Potassium	12 p.p.m.
Hydrochloric Acid (1 + 1)	0.2 ml. per 100 ml. solution
Ethyl Alcohol (95%)	20 ml. per 100 ml. solution

Instrument settings used for each of the three elements determined are tabulated below:

	<i>Calcium</i>	<i>Sodium</i>	<i>Potassium</i>
Oxygen pressure ( <i>in. of water</i> )	48	40	52
Air pressure ( <i>p.s.i.</i> )	28	28	28
Gas pressure ( <i>cm. n-butyl alcohol</i> )	3.5	3.5	3.5
Wavelength ( <i>mμ.</i> )	554	589	769
Slit width ( <i>mm.</i> )	0.3	0.05	0.2
Phototube used	ultraviolet	ultraviolet	red
Sensitivity:			
Switch	0.1	0.1	0.1
Knob	3 turns from counter clock-wise limit	2 turns from counter clock-wise limit	5 turns from counter clock-wise limit

Standard curves, fig. 5, were prepared as a guide for the choice of standards to be used in the determination of the unknowns. The solutions to be analyzed were handled in the following manner: Clean, dry, 5-ml. beakers were filled with the unknown solution and appropriate standard solutions. After the proper instrument settings were made and the spray chamber heated to operating temperature, the unknown was placed in position, atomized into the flame

and the relative intensity of the desired element determined. By referring this intensity to the standard curve, a choice of two standards was made such that one was of slightly lower, the other of slightly higher concentration than the unknown. The relative intensities of the ion in each of these two standards were quickly determined merely by replacing the beakers, balancing the circuit to the galvanometer null point and taking the intensity reading. Finally, the unknown was replaced on the instrument to check the value of its first reading, thereby checking the consistency of operation during the series of readings. If a variation greater than 0.2 scale division existed between the two readings of the unknown solution, the series of readings of the unknown and two standards was repeated until no variation existed between the first and second reading of the unknown. This manner of reading was preferred to that of averaging sev-

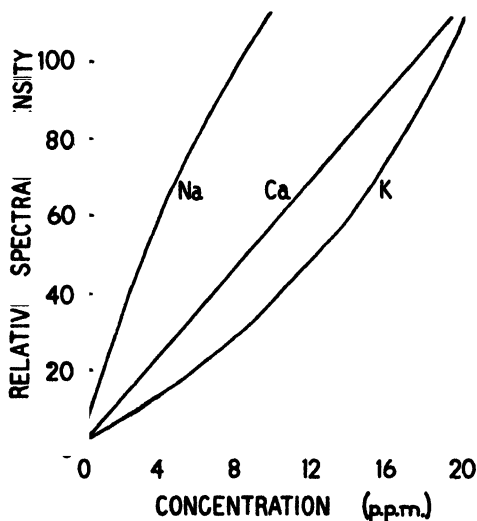


Fig. 5. Standard curves.

eral readings on each solution in that it lessened errors due to drifting and partial capillary clogging and generally required a shorter time. The remaining unknown solutions were handled in a similar manner to determine the same element. The instrument was reset for the determination of the second element and all unknown solutions determined. Finally, the group of unknowns was analyzed for the third element. The authors determined a group of twenty unknowns for three elements in duplicate in this fashion in 4 hr. including calculating time, but excluding time for sample and standard solution preparation.

Results were calculated in a similar way for the three elements determined. It was assumed that the standard curve over the small range between the two standards was a straight line in each case. This was true for calcium, but not strictly true for sodium or potassium (fig. 5). However, the error introduced

TABLE 1

*Per cent sodium, potassium and calcium in milk*

Sample	Sodium				Potassium				Calcium			
	By flame spectro- photometer method		By chemical method <sup>a</sup>		By flame spectro- photometer method		By chemical method <sup>b</sup>		By flame spectro- photometer method		By chemical method <sup>c</sup>	
1	0.0367	0.0371	0.0371	0.0376	0.111	0.112	0.112	0.112	0.111	0.112	0.112	0.112
2	0.0581	0.0577	0.0592	0.0590	0.106	0.106	0.107	0.106	0.106	0.106	0.107	0.106
3	0.0379	0.0386	0.0382	0.0379	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105
4	0.0409	0.0405	0.0396	0.0416	0.123	0.122	0.122	0.122	0.123	0.122	0.122	0.122
5	0.0405	0.0404	0.0404	0.0409	0.109	0.110	0.108	0.110	0.109	0.110	0.108	0.110
6	0.0496	0.0492	0.0496	0.0498	0.118	0.119	0.118	0.118	0.118	0.119	0.118	0.118
7	0.0358	0.0364	0.0360	0.0372	0.131	0.133	0.131	0.131	0.131	0.133	0.131	0.131
8	0.0443	0.0445	0.0437	0.0448	0.122	0.123	0.122	0.122	0.122	0.123	0.122	0.122
9	0.0430	0.0435	0.0493	0.0469	0.104	0.104	0.104	0.104	0.104	0.104	0.104	0.104
10	0.0404	0.0408	0.0413	0.0401	0.120	0.120	0.120	0.120	0.120	0.120	0.120	0.120

<sup>a</sup> Sodium was determined gravimetrically by precipitating with zinc uranyl acetate after the removal of interfering phosphate ion with zinc carbonate (1, 18).

<sup>b</sup> Potassium was determined volumetrically by a modification proposed by Bray (5) of the original Koninch (14) cobaltinitrite method.

<sup>c</sup> Calcium was determined by precipitation as the oxalate from a slightly acid solution followed by a permanganate titration of the precipitate. This method is given by Hillebrand and Lundell (12) and modified by Meloche, *et al.* (15).

by this assumption was negligible when the standards used for the determination were not too different in concentration so that the small segment of the curve could be assumed a straight line. Hence, if  $C_x$ ,  $C_1$ , and  $C_2$  are the concentrations of the unknown, the standard solution of lower concentration and the standard of higher concentration, respectively, and  $I_x$ ,  $I_1$ , and  $I_2$  are the corresponding relative intensities, then the concentration of the unknown is given by:  $C_x = C_1 + \frac{(C_2 - C_1)(I_x - I_1)}{(I_2 - I_1)}$ . Corrections for the effect of sodium and potassium on each other must be applied where necessary.

Table 1 shows results in duplicate of ten samples determined by the stated technique together with duplicate results determined chemically. The four results listed for each sample were determined from aliquots of the same solution of milk ash.

TABLE 2  
*Replicate determinations of the same sample*

Date	Calcium		Sodium		Potassium	
	Duplicates	Av.	Duplicates	Av.	Duplicates	Av.
Feb. 22	0.1180 0.1185	0.1182	0.0461 0.0467	0.0464	0.1416 0.1405	0.1410
23	0.1174 0.1194	0.1184	0.0459 0.0467	0.0463	0.1381 0.1387	0.1384
24	0.1169 0.1189	0.1179	0.0469 0.0463	0.0464	0.1366 0.1362	0.1364
25	0.1184 0.1181	0.1182	0.0459 0.0456	0.0458	0.1378 0.1378	0.1376
27	0.1200 0.1195	0.1198	0.0466 0.0466	0.0466	0.1386 0.1371	0.1378
28	0.1190 0.1190	0.1190	0.0462 0.0464	0.0463	0.1402 0.1420	0.1411
Mar. 1	0.1197 0.1198	0.1198	0.0467 0.0465	0.0466	0.1390 0.1385	0.1385
2	0.1189 0.1194	0.1192	0.0465 0.0460	0.0462	0.1374 0.1380	0.1377
3	0.1196 0.1190	0.1193	0.0466 0.0464	0.0465	0.1403 0.1382	0.1392
4	0.1189 0.1190	0.1190	0.0463 0.0464	0.0464	0.1398 0.1393	0.1396
Av.		0.1189		0.0464		0.1387

TABLE 3  
*Analysis of data in table 2*

	Calcium	Sodium	Potassium
Total sum of squares	0.000012	0.0000021	0.000075
Sum of squares between days	0.0000075	0.0000012	0.000041
Sum of squares within days	0.0000046	0.00000091	0.000034
Error within days	0.00000046	0.000000091	0.0000034
Error standard deviation on a particular day	0.00068	0.00030	0.0018
Per cent error	0.0057	0.0065	0.013
Number of replicate determinations necessary to give 99% accuracy	2	2	4

To determine reproducibility of this technique, one sample was chosen and the three elements determined in duplicate on each of 10 days. The results of this study are tabulated in table 2. The statistical analysis of data in table 2 is presented in table 3. The last line of table 3 gives the number of replicate determinations necessary to give 99 per cent accuracy. On the basis of duplicate determinations, potassium results were within  $\pm 2$  per cent of the amount present while sodium or calcium duplicates gave an accuracy of  $\pm 1$  per cent of the amount present. The average of the 20 replicates for each element was considered the amount present in the calculation of accuracy, since the exact amounts of the elements in the sample were unknown.

#### SUMMARY

A flame photometer technique suitable for single optical system instruments is described which gives excellent results for calcium, potassium and sodium in milk. The method consists essentially of determining the relative spectral intensity of the element in an unknown solution followed quickly by the determination of the intensity of the element in two standard solutions, one of slightly higher concentration, the other of slightly lower concentration than the unknown. The concentration of the unknown then is calculated by interpolation. The effects of extraneous ions upon the spectral intensity of the elements determined are minimized either by incorporating the influencing ions in the standard solutions at concentration levels similar to those found in the unknown solutions or by using appropriate predetermined numerical corrections.

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# BLOOD PLASMA VITAMIN A AND CAROTENE OF DAIRY CALVES TREATED WITH SULFONAMIDES<sup>1,2</sup>

MAGNAR RONNING AND C. B. KNOTT

*The Pennsylvania Agricultural Experiment Station, State College*

## INTRODUCTION

The antagonistic action of sulfonamides toward certain nutrients and metabolites has been reported (8, 9, 10, 11, 12, 18). These antagonistic effects apparently have not been considered complicating factors in the therapeutic use of drugs. A review of the available literature reveals that information concerning the effects of sulfonamide administration upon the normal utilization and physiological function of vitamin A, carotene and other compounds in the young calf is lacking. Lundquist and Phillips (12) found no apparent effect of sulfasuxidine administration on the blood vitamin A and carotene levels of calves. Further investigation with some of the commonly used drugs appeared to be desirable.

It has been demonstrated that calves at birth have a very limited reserve of vitamin A and that supplying an ample amount early in life is important (2, 4, 14, 15). A number of investigators have shown the importance of adequate supplies of vitamin A for proper growth and development and for resistance to diseases, particularly scours and pneumonia (6, 16, 17, 19, 22). It appeared to be advisable, should any indication of a disturbance of the normal vitamin A metabolism of a young calf due to administration of sulfonamides be demonstrated, that protective measures be undertaken in the routine raising of these animals. It was because of these and related problems that the present study was undertaken.

## EXPERIMENTAL PROCEDURE

Experiments were designed to ascertain the effects of the administration of sulfathaladine, sulfamerazine and sulfathiazole on the blood plasma vitamin A and carotene levels of young dairy calves. Male Holstein calves were procured from various Pennsylvania State institutional herds for the study. The calves were managed under controlled conditions for 1 wk. prior to the observation period to allow for stabilization of the animals.

The calves were quartered in individual pens equipped with a waterbowl, hay rack and feed trough, in an artificially heated and ventilated experimental calf barn. The rations consisted of 8 lb. of Holstein herd milk fed twice daily, with

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<sup>2</sup> Authorized for publication as paper no. 1579 in the Journal series of The Pennsylvania Agricultural Experiment Station.

U.S. no. 1 timothy hay and the following calf starter available *ad libitum*: Ground yellow corn, 406.5 lb.; wheat bran, 300 lb.; crushed oats, 400 lb.; linseed oil meal, 140 lb.; soybean oil meal, 280 lb.; dehydrated alfalfa meal, 140 lb.; cane molasses, 100 lb.; dried skim milk, 100 lb.; dried corn distillers' solubles, 100 lb.; irradiated yeast (type 9F), 0.5 lb.; dicalcium phosphate, 10 lb.; ground limestone, 10 lb.; iodized salt, 10 lb.; cobalt sulfate, 2 g.; and vitamin A feeding oil (2,724,000 U.S.P. units per lb.), 3 lb.

Twenty apparently normal calves were subdivided into four comparable groups (groups I, II, III and IV) and treated respectively with sulfathaladine, sulfamerazine and sulfathiazole and the last group maintained as controls. The experiments were begun when the calves were 12 days of age. Forty milliliters of blood for vitamin A and carotene analysis were drawn from the jugular vein daily, 3 hr. after the morning feeding, for 13 consecutive days.

The morning of the third day, when the calves were 14 days of age, sulfonamide administration was begun. A standard dosage was administered orally to all the calves of the treated groups as follows: 10 g. after the morning bleeding on the fourteenth day, followed by three 5-g. administrations at 12 hr. intervals. Approximately 6 ml. of blood were removed 3, 6 and 12 hr. after the first administration of the drug for sulfonamide determinations. Thereafter, the blood level of the drug was determined daily. When the calves reached the age of 28 days, the procedure was repeated on each calf with the same type and dose of sulfonamide as used previously. Untreated controls were similarly fed, managed and studied.

The carotene and vitamin A determinations were made using a combination of methods by Moore (13) and Kimble (5) as modified by Knodt (7). Sulfonamide blood levels were determined according to the Bratton-Marshall method (1). All determinations were colorimetric and the per cent transmission was measured with an Evelyn photoelectric colorimeter (3).

## RESULTS

The effects of the administration of sulfathaladine, sulfamerazine and sulfathiazole upon the levels of blood plasma vitamin A and carotene are summarized in tables 1 and 2. A comparison of the mean blood plasma levels for the 3 days prior to administration with those for the ninth, tenth and eleventh days after initial administration has been made. Whereas the blood plasma vitamin A of the control calves decreased 3.31  $\mu\text{g.}$  per 100 ml. during the first trial, changes of  $-0.01$ ,  $-2.67$  and  $+2.67$  were observed in the groups treated with sulfathaladine, sulfamerazine and sulfathiazole, respectively.

In the second trial, all groups of calves exhibited an increase in the mean blood plasma vitamin A levels, being more marked in the case of the sulfonamide-treated calves. The sulfathaladine-treated calves reacted similarly to those of the control group, but treatment with sulfamerazine and sulfathiazole resulted in greater increases in plasma vitamin A as compared to the controls. The greatest increase occurred with the administration of sulfamerazine.

Somewhat similar results were obtained with respect to the effects of those sulfonamides studied upon the levels of blood plasma carotene. With the exception of the calves constituting the sulfamerazine-treated group, the greatest decline in plasma carotene was exhibited by the control group. The decline observed in the case of group II might be attributed to the extremely high initial blood plasma carotene level of this group. In the second trial, all the sulfonamide-treated groups showed much greater increases in the carotene blood plasma

TABLE 1

*Mean daily blood plasma vitamin A concentrations of each group of calves ( $\mu\text{g./100 ml.}$ )*

Day of administration	Group I sulfathaladine	Group II sulfamerazine	Group III sulfathiazole	Group IV control
First trial				
- 1	11.03	17.91	10.85	14.68
0	10.68	18.25	11.50	15.91
1	9.68	15.57	10.86	14.46
2	8.92	15.57	10.29	13.38
3	9.01	14.07	10.47	13.57
4	10.20	13.67	11.23	13.13
5	10.41	14.24	12.96	13.46
6	10.16	14.91	12.72	12.80
7	9.75	14.82	12.27	12.49
8	9.63	16.74	11.66	12.10
9	9.91	15.32	14.30	12.52
10	10.27	15.08	14.27	11.30
11	11.16	13.30	12.64	11.30
$\bar{X}$	10.06	15.34	12.00	13.16
Change <sup>a</sup>	- 0.01	- 2.67	+ 2.67	- 3.31
Second trial				
- 1	11.94	11.19	11.35	10.04
0	9.52	9.67	11.30	10.83
1	9.26	8.95	10.30	10.66
2	9.27	9.55	10.60	10.77
3	9.52	11.07	9.40	10.13
4	10.33	12.71	11.88	10.20
5	11.08	12.49	12.88	11.33
6	10.91	10.51	12.57	10.32
7	11.30	11.77	13.35	10.69
8	11.30	12.08	12.02	10.87
9	10.45	11.94	12.32	11.53
10	10.99	12.60	12.85	11.23
11	11.22	11.80	12.49	10.20
$\bar{X}$	10.55	11.26	11.79	10.68
Change <sup>a</sup>	+ 0.65	+ 2.17	+ 1.57	+ 0.48

<sup>a</sup> A comparison of the -1, 0 and 1 with the 9, 10 and 11 d. of the trial.

levels than the control groups. It must be emphasized that in certain instances there were large variations in the response of the individual calves.

A summary of the free and total blood levels of the various sulfonamides used is presented in table 3. The highest levels were observed on the third day of administration in all cases. Sulfamerazine apparently was the most readily absorbed of the sulfonamides, while sulfathiazole was retained for a longer period. Sulfathiazole also exhibited the greatest degree of acetylation, as indicated by difference in the amount of the free and the total form of the drug

found in the blood. Although sulfathaladine was not absorbed to a very large extent, it apparently was acetylated at a rather high rate. Nearly all of the sulfamerazine detected in the blood was in the free form. In some instances, where low concentrations of blood sulfamerazine were observed, the difference between free and total sulfamerazine values was less than variations which might reasonably be attributed to laboratory techniques.

There was an increased absorption noted in the case of all the sulfonamides during the second trial and sulfamerazine and sulfathiazole apparently were

TABLE 2  
*Mean daily blood plasma carotene concentrations of each group of calves ( $\mu\text{g./100 ml.}$ )*

Day of administration	Group I sulfathaladine	Group II sulfamerazine	Group III sulfathiazole	Group IV control
First trial				
- 1	11.46	35.39	9.13	29.48
0	10.69	34.12	8.36	26.46
1	11.07	29.73	7.77	24.59
2	9.13	26.68	7.77	21.62
3	11.08	23.26	7.97	20.90
4	10.69	19.46	8.16	21.74
5	9.52	17.88	9.91	20.07
6	10.10	17.90	11.27	22.37
7	9.72	18.46	10.88	21.21
8	9.52	19.04	10.32	20.62
9	10.30	19.04	10.49	20.40
10	10.49	17.68	10.30	19.63
11	10.30	17.29	12.44	20.21
$\bar{X}$	10.31	22.76	9.60	22.25
Changes <sup>a</sup>	- 0.71	- 15.08	+ 2.66	- 6.76
Second trial				
- 1	14.38	24.21	10.50	17.10
0	13.99	24.21	10.30	15.94
1	13.60	20.99	10.30	15.16
2	13.99	19.08	11.08	14.57
3	14.77	21.57	11.27	16.71
4	15.74	24.79	12.05	18.07
5	17.29	27.12	14.77	16.13
6	17.29	27.12	14.57	15.54
7	18.07	29.59	14.77	16.52
8	19.43	27.31	14.38	15.94
9	19.82	26.90	15.16	17.29
10	21.18	27.70	15.16	18.10
11	22.35	28.59	14.77	17.29
$\bar{X}$	17.07	25.32	13.01	16.49
Changes <sup>a</sup>	+ 7.13	+ 4.59	+ 4.66	+ 1.49

<sup>a</sup> A comparison of the - 1, 0 and 1 with the 9, 10 and 11 d. of the trial.

absorbed at a higher initial rate. Sulfathaladine, however, exhibited a lower rate of initial absorption during this period as compared to the first trial.

#### DISCUSSION

It appears that under the conditions of these experiments, calves which had been treated with sulfonamides maintained blood plasma concentrations of vitamin A and carotene at comparable levels with those observed in the blood of control animals.

The relative rates and degrees of absorption of the various sulfonamides were found to be in agreement with the reports of other investigations (20, 21).

### SUMMARY

Observations were made to determine possible effects of sulfonamide administration upon the blood plasma vitamin A and carotene levels of young calves.

On the basis of these data, it does not appear that sulfonamide therapy has any detrimental effects on the normal vitamin A and carotene metabolism of calves when the drugs are administered as recommended.

TABLE 3

*Mean free and total sulfonamide blood concentrations of each group of calves from 3 hr. after first administration to end of period (mg./100 ml.)*

Day	Hour	Group I sulfathaladine		Group II sulfamerazine		Group III sulfathiazole	
		Free	Total	Free	Total	Free	Total
First trial							
1	3	0.14	0.23	0.64	0.62	0.58	0.63
	6	0.31	0.56	2.69	2.61	1.44	1.69
	12	0.53	0.92	4.70	4.72	1.93	2.37
2		0.58	1.09	8.14	8.49	2.88	3.57
3		0.68	1.24	12.14	12.81	4.09	6.19
4		0.62	1.05	11.87	12.77	2.86	4.35
5		0.36	0.68	9.65	10.23	1.95	4.30
6		0.27	0.41	6.01	10.75	0.82	2.04
7		0.09	0.18	2.73	2.84	1.03	3.87
8		0.05	0.10	1.40	1.49	0.55	2.14
9		0.06	0.14	1.18	1.29	0.50	2.08
10		0.04	0.12	0.23	0.24	0.10	0.30
11		0.01	0.04	0.28	0.31	0.18	1.09
Second trial							
1	3	0.02	0.07	1.88	2.10	0.84	0.98
	6	0.10	0.25	6.09	6.03	1.51	1.88
	12	0.36	0.69	8.38	8.33	1.99	2.91
2		0.66	1.11	10.70	9.91	3.66	5.24
3		1.20	1.83	14.49	13.80	5.22	8.19
4		0.88	1.40	8.24	8.87	3.64	6.91
5		0.24	0.51	2.82	4.17	2.86	6.20
6		0.10	0.26	0.62	1.65	2.48	6.00
7		0.11	0.23	0.16	0.44	1.92	5.03
8		0.06	0.63	0.06	0.10	1.41	4.39
9		0.06	0.16	0.00	0.00	0.92	3.42
10		0.01	0.10	0.00	0.00	0.72	3.08
11		0.03	0.14	0.00	0.00	0.43	2.61

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# MOTILITY OF BOVINE SPERMATOZOA IN BUFFERED WHOLE EGG

H. O. DUNN AND R. W. BRATTON

*Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry,  
Cornell University, Ithaca, New York*

The cost of ingredients and labor going into the preparation of yolk extenders for bovine semen is considerable when large quantities are required. Swanson (8) has reported satisfactory results with an extender consisting of three parts citrate buffer and one part egg yolk. Thus, substantial savings appear to be possible by reducing the amount of egg yolk used. If the whole egg (yolk plus albumen) could be utilized, even more savings would be expected, since the volume of yolk plus albumen from an egg is approximately three times the volume of the yolk itself.

At the time that Phillips and Lardy (4) reported the use of buffered liquid egg yolk as a satisfactory medium in which to store bovine spermatozoa, they also reported that egg yolk or egg albumen used alone was not satisfactory. Furthermore, they reported the optimum pH range for the storage of bovine spermatozoa to be 6.7 to 6.8 and the pH of egg yolk to be approximately 6.0. Therefore, a suitable buffer to adjust the pH of the yolk was indicated. The fact that whole egg and egg albumen have pH values of 7.5 to 7.8 and 8.0 to 9.0, respectively, (2, 5) suggests that the unsatisfactory results reported from the use of egg white alone (4) may have been, in part, a consequence of its pH. However, to date, the use of whole egg with a suitable buffer has not been reported. This paper reports the results of laboratory investigations undertaken to utilize liquid whole egg instead of egg yolk in extenders for bovine semen.

## EXPERIMENTAL

The liquid whole egg was prepared by emptying the contents of alcohol-cleaned eggs into a sterile beaker, removing the chalazae with sterile forceps and mixing the whole egg material for 2 min. in a Waring blender. After allowing the blended whole egg material to settle from the foam, it was poured off and immediately mixed with the buffer in the required proportions.

Initially, spermatozoan motility studies were conducted comparing the standard 2.9 per cent citrate-sulfanilamide-yolk (2.9 CSAY) extender (1) and an experimental extender composed of equal parts of 2.9 per cent citrate-sulfanilamide buffer (1) and blended liquid whole egg. Twenty samples of bovine semen were extended at the rate of 1:100 in these two extenders and stored at 5° C. in 3-ml. tubes filled to capacity. At the end of 2 days storage, 44 per cent of the spermatozoa were motile in the 2.9 CSAY but only 11 per cent were motile in the 2.9 per cent citrate-sulfanilamide-whole egg extender (2.9 CSAYWE). This difference was reasonable in view of the fact that the pH of the yolk extender was 6.66, while the pH of the whole egg extender was 7.82.

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On the basis of these initial observations, a series of citrate-sulfanilamide-whole egg extenders was prepared in which the pH was adjusted by the use of acetic, lactic, succinic and hydrochloric acid. In addition to these common acids, a sulfonamide with acidic properties, succinylsulfathiazole (sulfasuxidine), was used to lower the pH of the whole egg extender and, at the same time, substitute for sulfanilamide. In these extenders the proportion of buffer to whole egg was changed from 1:1 to 3:1, but the concentration of citrate and sulfonamide was maintained at the same levels as in the 2.9 CSAY, namely, 1.45 per cent sodium citrate dihydrate and 300 mg. per cent sulfonamide. Therefore, an extender having a 3:1 ratio of buffer to whole egg required a buffer containing 1.93 per cent citrate and 400 mg. per cent sulfonamide. Twenty samples of fresh semen were extended at rates of 1:100 in these acidified whole egg extenders and compared with those same samples extended in the standard 2.9 CSAY (1). The initial pH of each extender and the per cent of motile spermatozoa at 0, 1, 2, 3 and 4 days of storage at 5° C. are shown in table 1.

TABLE 1

*Per cent of motile spermatozoa in extenders containing whole egg acidified with different acids and stored at 5° C.  
(Averages of 20 ejaculates from 14 bulls)*

Extenders <sup>a</sup>	Acid used	pH of extender	Days stored				
			0	1	2	3	4
2.9 CSAY	None	6.71	56	47	42	34	32
1.9 CSAWE	None	7.88	46	10	4	1	1
“	Acetic	6.58	54	41	33	29	26
“	Lactic	6.81	58	43	37	32	27
“	Succinic	6.66	56	41	36	36	32
“	HCl	6.60	58	46	39	33	30
1.9 CSSWE	None	6.67	59	49	44	37	35

<sup>a</sup> 2.9 and 1.9 represent the percentages of sodium citrate dihydrate in the buffer; C = sodium citrate dihydrate; SS = sulfasuxidine; SA = sulfanilamide; Y = egg yolk; WE = whole egg (yolk and albumen).

From these storage data it was obvious that adjustment of the pH was of primary importance in the utilization of whole egg. The combination of three parts of 1.93 per cent citrate containing 0.4 per cent sulfasuxidine and one part of whole egg (1.9 CSSWE) appeared to be as satisfactory for spermatozoan motility as the standard 2.9 CSAY. Since the use of the common acids for pH adjustment required one more step in buffer preparation, the use of sulfasuxidine was considered desirable.

To avoid the slight hypotonicity accompanying the use of the 1.93 per cent citrate-sulfasuxidine buffer, the 2.9 per cent citrate buffer containing 0.4 per cent sulfasuxidine was used. Each buffer was mixed in a ratio of three parts buffer to one part whole egg and compared with the standard 2.9 CSAY. Twenty fresh semen samples were extended at the rate of 1:100 in these three extenders and stored at 5° C. for 4 days. The initial pH of the extenders and the per cent of motile spermatozoa during storage are shown in table 2. During the first day



or two of storage, the 2.9 CSSWE was slightly superior to both the 1.9 CSSWE and the 2.9 CSAY control.

Another sulfonamide, succinylsulfanilamide, a derivative of sulfanilamide having acidic properties, was used to replace the succinylsulfathiazole in the 2.9 CSSWE formula. Motility of the spermatozoa appeared to be as satisfactory in the whole egg extender containing the succinylsulfanilamide (2.9 CSSAW) as in the 2.9 CSSWE or the 2.9 CSAY.

During the course of these storage studies, it was observed that the blended whole egg and those whole egg extenders with a high pH changed within a few hours from a light yellow to a dark brown color. These color changes may have been caused by enzymatic reactions at the high pH levels (3), since the whole egg extenders adjusted to a pH of 6.6 to 6.8 retained their initial light yellow color, even when stored with spermatozoa for 30 days. Combinations of liquid whole egg with phosphate buffers have not been satisfactory because of the precipitates formed.

TABLE 2

*Per cent of motile spermatozoa in yolk and whole egg extenders when stored at 5° C.  
(Averages of 20 ejaculates from 18 bulls)*

Extenders <sup>a</sup>	pH of extender	Days stored				
		0	1	2	3	4
2.9 CSAY	6.72	54	52	46	43	38
2.9 CSSWE	6.75	65	56	49	37	34
1.9 CSSWE	6.68	52	44	44	40	38

<sup>a</sup> See footnote table 1.

#### DISCUSSION

The whole egg extenders cost approximately 25 per cent as much as the standard yolk extenders. Other advantages were the small amount of time required for preparation, the ease with which glassware coming in contact with them was cleaned and the more distinct appearance of the spermatozoa during microscopic examinations. Settling out, which is a problem in the mixing of samples for microscopic examination or for insemination, was less pronounced in the 1.9 CSSWE and the 2.9 CSSWE than in the 2.9 CSAY. This may be explained by the fact that sulfasuxidine is more soluble than sulfanilamide and does not crystallize out readily during storage at 5° C. in the whole egg formulae. It also was observed that, in contrast to the dark brown color developed by the citrate buffers containing sulfanilamide when exposed to sunlight (6), those buffers containing sulfasuxidine developed only a light brown color.

From the results of these studies it appears that citrate-sulfasuxidine buffered liquid whole egg may be used as an extender for bovine semen if it does not impair the fertility of the spermatozoa. Field trials to test the fertility of spermatozoa stored in citrate-sulfasuxidine buffered liquid whole egg have been conducted, the results of which are to be reported in an accompanying paper.

## SUMMARY

Spermatozoan motility at 5° C. was as satisfactory in extenders composed of three parts of a 2.9 per cent sodium citrate dihydrate buffer containing 0.4 per cent of succinylsulfathiazole or 0.4 per cent succinylsulfanilamide and one part of liquid whole egg as in the standard 2.9 per cent citrate-sulfanilamide-yolk extender.

The costs of the whole egg extenders were approximately 25 per cent of that of the yolk extender. The former could be prepared more quickly and glassware coming in contact with them was much easier to clean. The spermatozoa were more visible and more active in the whole egg extenders, thereby facilitating microscopic examinations.

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# FERTILITY AND MOTILITY OF BOVINE SPERMATOZOA IN BUFFERED WHOLE EGG EXTENDERS

H. O. DUNN, R. W. BRATTON AND W. J. COLLINS

*Laboratory of Animal Breeding and Artificial Insemination*

*Department of Animal Husbandry, Cornell University, Ithaca, New York*

In previous reports from this laboratory, Dunn and Bratton (2, 3) presented preliminary data showing that satisfactory motility and fertility of bovine spermatozoa could be obtained with a citrate-sulfasuxidine buffered liquid whole egg extender. The present paper reports results of more extensive investigations on the fertility and motility of bovine spermatozoa in this type of extender.

## EXPERIMENTAL

Two field trials were conducted, the first from December 28, 1948, to January 11, 1949, and the second from May 16 to June 9, 1949. In the first trial each of 69 ejaculates from 37 Holstein bulls was split two ways. One portion was extended in 2.9 per cent citrate-sulfanilamide-yolk (2.9 CSAY) and the other in 1.9 per cent citrate-sulfasuxidine-whole egg (1.9 CSSWE). In the second trial each of 145 ejaculates from 48 bulls, 31 Holsteins and 17 Guernseys, was divided into four portions and each portion extended in one of the following extenders: 3.5 CSAY, 2.9 CSAY, 2.9 CSSWE, and 1.9 CSSWE.

The composition of the buffers and extenders are shown in table 1. The buffers were prepared once a week and the extenders during the afternoon of the day prior to their use.

TABLE 1  
*Composition of buffers and extenders*

Extenders <sup>a</sup>	Concentration of citrate and sulfonamides in buffers			Proportion of buffer to egg material		Concentration of citrate and sulfonamides in extenders			
	C	SA	SS	Y	WE	C	SA	SS	pH
	(%)	(%)	(%)			(%)	(%)	(%)	
3.6 CSAY .	3.60	0.60		1:1		1.45	0.30		6.78
2.9 CSAY .	2.90	0.60		1:1		1.45	0.30		6.76
2.9 CSSWE .	2.90		0.40		3:1	2.175		0.30	6.75
1.9 CSSWE	1.93		0.40	..	3:1	1.45		0.30	6.70

<sup>a</sup> 1.9, 2.9 and 3.6 represent the per cent of sodium citrate dihydrate in the buffer. C = citrate; SA = sulfanilamide; SS = sulfasuxidine; Y = egg yolk; WE = whole egg.

In both trials each portion of each ejaculate was partially extended (1:4) in its respective extender immediately after collection, cooled slowly from approximately 30 to 5° C. during a period of 55 min. and then made up to the final volume required with cold (5° C.) extender. Only semen samples initially con-

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taining  $500 \times 10^6$  or more spermatozoa per ml. and 50 per cent or more motile spermatozoa were used for insemination. Final extension rates averaged 1:68 in the first trial and 1:86 in the second trial, the average number of motile spermatozoa per milliliter of extended semen being  $17 \times 10^6$  and  $14 \times 10^6$ , respectively, in the two trials. Microscopic examinations of all extended semen samples were made at intervals of 0, 1 and 3 days of storage at 5° C.

The fertility of the semen was based on 60- to 90-day non-returns to first and second service cows and expressed as per cent non-returns. Analysis of variance (7) was used to test the statistical significance between the means, the ejaculate  $\times$  extender sub-class percentages being the experimental units.

#### RESULTS AND DISCUSSION

In table 2 are the estimated percentages of motile spermatozoa during storage at 5° C. in the different extenders. The per cent of motile spermatozoa compared favorably with previous findings (2, 3). The rate of progressive movement of the spermatozoa was definitely superior in the whole egg extenders. While theoretically the 1.9 CSSWE was slightly hypotonic (6), the motility of spermatozoa in this extender appeared to be as satisfactory as in the 2.9 CSSWE and the yolk extenders.

TABLE 2

*Average per cent of motile spermatozoa in citrate-yolk and citrate-whole egg extenders when stored at 5° C.*

	Days of storage	Extenders*			
		3.6 CSAY	2.9 CSAY	2.9 CSSWE	1.9 CSSWE
1st trial	0		61		
(av. of 69 ejac. from 37 bulls)	1		56		
	3		49		
2nd trial	0	66	64	69	
(av. of 145 ejac. from 48 bulls)	1	60	60	64	
	3	53	56	51	

\* See footnote table 1.

In table 3 are the total number of first- and second-service cows inseminated in the two trials and the per cent 60- to 90-day non-returns to these cows. In both trials the per cent non-returns for the first- and second-service cows combined averaged slightly higher for the 2.9 CSAY than for the 1.9 CSSWE extender. In the second trial the per cent non-returns averaged slightly higher for the 2.9 CSAY than for the 2.9 CSSWE. On the other hand, the average non-return percentages for first- and second-service cows combined were slightly higher than they were for the 3.6 CSAY formula. Yet, none of the differences between the means for the different extenders were statistically significant ( $P < 0.05$ ).

In the two trials, 10,923 first- and second-service cows were inseminated with the CSAY formulae and 11,289 with the CSSWE formulae. On the basis of the 60- to 90-day non-returns, the yolk formulae (3.6 CSAY and 2.9 CSAY) averaged

62.3 per cent and the whole egg formulae (1.9 CSSWE and 2.9 CSSWE) averaged 61.6 per cent. Therefore, it appears that 2.9 CSSWE may be used as an extender for bovine semen with the expectation of fertility results practically as good as those obtained by the use of 2.9 CSAY extender.

In view of the favorable results on fertility levels reported by Almquist (1), Easterbrooks *et al.* (4) and Foote *et al.* (5) when penicillin and streptomycin have been used in the citrate-sulfanilamide-yolk extender, the question arises as to whether or not the addition of these antibacterial agents to the whole egg extender will be accompanied by similar increases in fertility. Fertility studies are being initiated to compare yolk and whole egg extenders containing penicillin, streptomycin and a sulfonamide.

TABLE 3

*Fertility data*

(Average per cent 60-90-day non-returns to 1st and to 1st and 2nd service cows combined)

	Extenders <sup>a</sup>							
	3.6 CSAY		2.9 CSAY		2.9 CSSWE		1.9 CSSWE	
	No. of serv.	N. R. <sup>b</sup>	No. of serv.	N. R.	No. of serv.	N. R.	No. of serv.	N. R.
	(%)		(%)		(%)		(%)	
1st trial:								
1st service cows			2,926	59.9			2,987	59.2
1st & 2nd service cows combined			4,144	57.6			4,336	56.6
2nd trial:								
1st service cows	2,543	64.4	2,614	66.7	2,657	66.1	2,549	64.3
1st & 2nd service cows combined	3,370	63.9	3,409	66.0	3,517	64.5	3,436	64.2

<sup>a</sup> See footnote table 1.

<sup>b</sup> N. R. = non-returns.

## SUMMARY

By means of the split sample technique, comparisons were made between the standard 2.9 and 3.6 per cent citrate-sulfanilamide-yolk extenders and whole egg extenders composed of three parts of either a 1.9 or a 2.9 per cent citrate buffer, containing 400 mg. per cent of succinylsulfathiazole and one part of blended liquid whole egg (yolk + albumen).

On the basis of approximately 11,000 first- and second-service cows inseminated artificially with each of these two types of extenders, the 60- to 90-day non-returns averaged 62.3 per cent for the citrate-sulfanilamide-yolk formulae and 61.6 per cent for the citrate-succinylsulfathiazole-whole egg formulae. Spermatozoan motility was satisfactory in both types of extenders.

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# ADAPTATION OF THE TYRAMINE METHOD TO ROUTINE CHEESE ANALYSIS<sup>1</sup>

F. V. KOSIKOWSKY AND A. C. DAHLBERG

*Department of Dairy Industry, Cornell University, Ithaca, N. Y.*

In a previous paper (3) the authors presented data on the tyramine concentration of various cheeses. The method used was one applied from the studies of Bellamy and Gunsalus (1) on bacterial metabolism. Although the results obtained on cheese were accurate, the method was not conducive to large-scale operations. This was due to pre-extraction of the cheese fat and to formation of emulsions in the extraction tube produced by rapid rates of extraction. As a result, slow extraction rates were used with 48 hr. being considered average extraction time for a sample of cheese.

Recently several changes have been introduced to speed up and simplify the method. These changes include elimination of the extraction of fat, greater dilution of cheese solutions to minimize emulsion formation and changes in quantities of cheese and color reagents. The modified method for cheese is presented in this paper.

## PROCEDURE

**Reagents<sup>2</sup>.** (a) Sodium carbonate solution (2 per cent). Dissolve 2 g. of sodium carbonate (anhydrous) in water and make up to 100 ml. (b) Sulphuric acid solution (*M*/50). Dilute 1.1 ml. of concentrated sulphuric acid to 1 l. (c) Phenolphthalein (1 per cent). Dissolve 1 g. phenolphthalein powder in a small quantity of 95 per cent alcohol and bring to 100 ml. with alcohol. (d) Ethyl ether, USP. (e) Acetic acid (95 per cent). (f) Mercuric sulphate-sulphuric acid solution. Dissolve 20 g. of mercuric sulphate in 190 ml. of water to which 10 ml. of concentrated sulphuric acid have been added. (g) Sodium nitrite 1.5 per cent. Dissolve 750 mgm. of  $\text{NaNO}_2$  in 50 ml. of water. Use fresh daily. (h) Whatman filter paper, no. 42, 15 cm. (i) Apparatus.<sup>3</sup> Refer to Kosikowsky and Dahlberg (3).

**Preparation of sample.** Using a mortar, 2.5 g. of cheese were ground to a paste with a small amount of warm (50° C.) water. Later, more warm water was added and the mixture was transferred quantitatively to a 250-ml. volumetric flask where the level of water was brought to approximately two-thirds full. This mixture then was heated in a water bath to 85° C. and, without holding, was cooled to about 25° C. The flask was filled to its mark with water, stoppered and inverted several times. The mixture was filtered through large fluted

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<sup>1</sup> This investigation was aided by a grant from the National Cheese Institute.

<sup>2</sup> Unless otherwise specified, C.P. chemicals and distilled water were used.

<sup>3</sup> The complete apparatus can now be obtained from Will Corporation, Rochester, N. Y. Receiver tube will have a 6-ml. graduation on lower portion.

Whatman no. 42 paper and when enough of the relatively clear filtrate was obtained, 25-ml. samples were transferred to extraction tubes.

*Extraction of cheese filtrate.* The 25 ml. of cheese filtrate were made slightly alkaline by adding from 0.2 to 0.8 ml. of 2 per cent sodium carbonate solution (No phenolphthalein actually was added to the sample used in the determination, but the amount of alkali required was determined on a duplicate sample of cheese filtrate to which two drops of phenolphthalein were added). Five ml. of *N*/50 sulphuric acid were placed in the receiver tube, the glass thimble placed in the extractor tube and ethyl ether carefully added to both tubes. The two sections then were connected by means of a cork and attached to a water condenser. Immersion of the ends of the extractor tubes in an oil bath at 65 to 70° C. provided sufficient heat to force extraction at the rate of 70 to 80 drops per min. and the average cheese generally was complete in 18 to 24 hr. Pre-extraction of the fat under acid condition was not required in this method, as the major portion of the fat was removed by filtration in preparing the sample and the sample also was more dilute.

After extraction, the receiver tube was disengaged, placed into the oil bath and the ether was carefully boiled off. The remaining acid solution was cooled to 25° C., the volume brought up to the 6-ml. graduation lines with dilute sulphuric acid and agitated.

*Development and measurement of color.* Two ml. of the acid solution containing the tyramine were pipetted into a colorimeter tube (calibrated 18 × 150 ml. pyrex test tube). Three ml. of acetic acid were added, followed by 2 ml. of the mercuric sulphate-sulphuric acid reagent and the tube was well agitated. This mixture was heated for 3 min. in boiling water and cooled to room temperature. The tube was placed in a Coleman no. 11 spectrophotometer and was read at 500  $\gamma$  against a reagent blank set at  $G = 100$ . After the turbidity reading ( $L_1$ ), if any, was recorded, 0.2 ml. of fresh 1.5 per cent sodium nitrite was added and well mixed. After 15 min. at room temperature, the tube was read against a reagent blank. This second reading was labeled  $L_2$ .

The quantity of tyramine can be calculated by using a constant or it can be observed by comparison with a standard curve. Both methods were illustrated by the authors in their previous paper (3). It should be pointed out here that in making calculations the 25 ml. of cheese filtrate contains 0.25 g. of cheese and that as 2 ml. out of 6 ml. are tested, the factor to convert back to the tyramine-per-gram basis would be 12.

#### EXPERIMENTAL RESULTS

A number of cheddar cheeses were analyzed for tyramine by the method previously used by the authors and by the method presented here. Table 1 showing the results on ten of these cheeses indicates that the recovery of tyramine from cheese is not reduced by the introduction of these changes.

The possibility of extracting dry cheese, using Soxhlet type fat extraction units, also was explored. Extractions of this nature should be a great deal faster and simpler. Bailey-Walker extractors with a variety of siphon cups and



extraction thimbles were used. A series of cheeses was ground with either anhydrous sodium carbonate or anhydrous sodium sulphate in amounts sufficient to bind all the water in the cheese. The pH of the dry cheese with sodium carbonate had the correct pH for tyramine extraction, while the cheese mixed with sodium sulphate was on the slightly acid side. Upon extraction with ether, using various types of glass and paper extracting thimbles, both cheeses gave large amounts of color. As tyramine would not have been extracted from an acid cheese, it was apparent that through some mechanical defect either tyramine, tyrosine or some water soluble protein was coming over into the test solution. This was confirmed further by the fact that when cheeses extremely low in

TABLE 1

*The tyramine concentration of cheddar cheese using the original tyramine method and the modified tyramine method*

Cheeses	Original tyramine method (direct extraction of cheese)	Modified tyramine method (extraction of water extract)
	( $\gamma$ tyramine/g. cheese)	( $\gamma$ tyramine/g. cheese)
1	184	188 178
2	440	459 426
3	294	260 260
4	701	718 685
5	82	76 78
6	308	311 313
7	687	718 695
8	104	102 102
9	441	400 415
10	874	880 880

tyramine and high in tyrosine were analyzed, there was a carry-over. When applied to wet extraction, using the regular extraction apparatus, this condition did not exist. It was apparent that dry extraction of cheeses for tyramine under these conditions was not feasible.

#### DISCUSSION

Several changes in procedure have been advocated to improve the method of determining tyramine in cheese. The tendency for emulsions to form in the extraction tube and thus hamper the removal of tyramine has been reduced but not eliminated. For certain types of cheddar cheese these emulsions still persist and can only be handled with care as advocated earlier by the authors. The complete elimination of these emulsions would improve the method tremendously.

Attempts made in this laboratory to successfully eliminate this condition by the use of various extraction technics and by the use of protein precipitation agents have met with little success. The precipitating agent which showed the greatest promise in this approach, as pointed out by Gurdian (2), was zinc sulphate. However, although certain specific concentrations of zinc sulphate completely eliminated emulsion formation during the time required for running this test, there was a definite loss in recovery of tyramine which, though averaging only 5 per cent, was not constant.

In addition to the lessened tendency for emulsions to form, these changes have provided advantages of less handling of equipment, shorter operational time and easier computations. During the past year, over 200 samples were analyzed successfully using these changes in routine work.

In the last paper (3) on tyramine determination of cheese, sodium nitrite had been inadvertently printed as sodium nitrate. The latter, if used, will not produce color in the presence of tyramine. The present paper contains the corrected term, sodium nitrite ( $\text{NaNO}_2$ ).

#### SUMMARY

The determination of tyramine in cheese has involved a long tedious procedure. A number of changes have been made to shorten the method without loss of accuracy. These include elimination of initial fat extractions, greater dilution of cheese solutions to reduce emulsion formation, more rapid extraction and use of different quantities of cheese and reagents. These changes make this method more adaptable for routine analysis of cheese.

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# LIVE SPERMATOOZOA RELATIONSHIPS AND FERTILITY OF DAIRY BULL SEMEN<sup>1</sup>

EDWARD J. STONE,<sup>2</sup> JAMES E. JOHNSTON AND JOHN P. MIXNER

*New Jersey Agricultural Experiment Station, Sussex*

A criterion for selecting semen samples to be used for artificial insemination which has a high correlation with the fertilizing capacity of the semen would be of great value. For the differentiation of live and dead spermatozoa, Lasley *et al.* (3) proposed a staining method employing opal blue and water soluble eosin as the dyes. Lasley and Bogart (2) employed this differential stain in a study of artificial breeding in beef cattle. They reported a coefficient of correlation of +0.41\*\*<sup>3</sup> between the percentage of live spermatozoa and the motility rating of semen samples and a correlation of +0.83\*\* between fertility and the percentage of live spermatozoa in diluted semen following a 0° C. cold shock for 10 min. They also reported that semen samples containing less than 50 per cent live spermatozoa were of questionable fertilizing capacity, whereas samples containing 50 to 90 per cent live spermatozoa showed no differences in fertilizing capacity.

Madden *et al.* (4) have used the opal blue-eosin stain in other semen studies.

Schaeffer and Almquist (7, 8) reported on a differential stain employing aniline blue and eosin in the staining mixture. Coefficients of correlation between the percentage of live spermatozoa initially in semen samples and their fertilizing capacity were not sufficiently high for prediction purposes.

Mayer *et al.* (5) also have reported on a variation of the opal blue-eosin stain, substituting fast green FCF for the opal blue in the staining mixture.

The purpose of the experiments reported here was to establish further the relationships existing between certain semen characteristics (including the percentage of live spermatozoa in semen samples initially and following cold shock) and fertility, with a view to the use of these relationships as criteria for selecting semen samples for artificial insemination.

## METHODS

This study was made on semen collected from 19 dairy bulls (14 Holsteins and 5 Guernseys) in a stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex. Inseminations were made by the technicians of the Sussex County Cooperative Breeding Association, Inc., in their local cooperative. Data were collected on 236 semen samples, among which were 81

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<sup>2</sup> Now at the Dept. of Dairy Ind., North Dakota Agr. College, Fargo.

<sup>3</sup> Throughout this paper \* represents significance at the 5 per cent point and \*\* represents significance at the 1 per cent point.

from 14 bulls to which 20 or more first- and second-service cows were bred for a total of 2,753 inseminations. Fertility data are based on 60- to 90-day non-returns to service. Semen samples that were used for inseminating purposes were selected on the basis of initial motility rating and the previous breeding efficiency of the bulls.

The semen samples were collected by means of an artificial vagina and processed immediately. Spermatozoa concentration was determined turbidometrically (Salisbury *et al.*, 6) with a Klett-Summerson photoelectric colorimeter. Motility ratings were made initially on undiluted semen according to the procedure of Herman and Swanson (1) and Swanson and Herman (10). Motility ratings were made daily until motility ceased, both on the undiluted semen and on a sample of semen diluted one part to ten parts of an egg yolk-citrate diluter containing 0.3 per cent sulfanilamide. The storage temperature was 5° C. Using the fast green FCF-eosin Y differential stain (Mayer *et al.*, 5), an initial determination of the percentage of live spermatozoa was made on each semen

TABLE 1  
*Characterization of 236 semen samples from 19 bulls*

	Mean	Standard error	Standard deviation	Range	Coefficient of variation <sup>a</sup>
					(%)
Undiluted semen					
Sperm concentration ( <i>millions/ml.</i> )	1,388.84	31.00	476.23	542-3,025	34.28
Semen volume ( <i>ml.</i> )	4.33	0.10	1.56	1.6-12.7	36.03
Sperm per ejaculate ( <i>billions</i> )	6.17	0.27	4.09	1.5-27.3	66.29
Initial motility rating	3.27	0.03	0.54	2-5	16.51
Duration of "2" motility ( <i>hr.</i> )	123.60	3.35	51.60	12-324	41.75
Total duration of motility ( <i>hr.</i> )	197.13	5.40	83.18	36-612	42.20
Diluted semen					
Total duration motility ( <i>hr.</i> )	445.89	8.60	132.34	180-876	29.68
Initial live sperm (%)	67.06	0.77	11.85	29-90	17.67
Live sperm following cold shock (%)	53.96	0.98	15.13	11-83	28.04
Fertility (81 samples) (%)	66.53	1.18	10.73	41-86	16.13

<sup>a</sup> Based on total variation.

sample and the percentage of live spermatozoa was determined after subjecting the diluted semen to a 0° C. temperature for 10 min.

Data from this study were analyzed by Fisher's analysis of variance, as described by Snedecor (9).

#### RESULTS

A characterization of the 236 semen samples studied is presented in table 1. The means with standard errors, standard deviations and ranges for each semen character studied are included in this characterization.

An analysis of variance of the data indicated that highly significant differences among bulls existed for all semen characters studied (table 2).

Since sufficient data were collected in this study on the individual bulls, it was decided to present the correlation analysis on a "total," "between" and "within bull" basis. This represents an extension of the usual "total" and

TABLE 2  
*Analysis of variance of data on semen characteristics*

Variance source	Degrees of freedom	Mean square					
		Concentration	Volume	Total sperm	Undiluted semen		
					Initial motility	Duration of 2 motility	Duration motility
Total	235	226,793.5	2.42	16.73	0.29	2,662.46	6,919.73
Between bulls	18	1,433,430.1**	10.87**	75.12**	1.11**	5,799.44**	15,417.50**
Within bulls	217	120,098.18	1.72	11.89	0.22	2,402.24	6,214.84

	Degrees of freedom	Mean square			Degrees of freedom	Mean square
		Diluted semen				
		Duration motility	Initial live sperm	Live sperm, 0° C.		
			(%)	(%)		
Total	235	17,513.19	140.52	228.95	80	39.18
Between bulls	18	45,933.89**	409.94**	646.33**	13	296.35**
Within bulls	217	15,173.14	118.17	194.33	67	79.82

"grouped" data correlations presented in other studies of this type. Thus, data on all semen characters studied were correlated with data on initial percentage of live spermatozoa, percentage of live spermatozoa following the 0° C. cold shock and fertility (table 3). The coefficients of correlation show that, in most instances, the "between bull" correlations are higher than the "total" or the "within bull" correlations. Since there are fewer degrees of freedom involved in the "between bull" correlations, few of these achieve statistical sig-

TABLE 3

*Tabulation of total, between and within bull coefficients of correlation among various semen characteristics and fertility<sup>a</sup>*

Semen characteristics		Initial live sperm	Live sperm surviving 0° C.	Fertility
		(%)	(%)	
<i>Undiluted semen</i>				
Sperm concentration	Total	+ 0.07	- 0.05	+ 0.23*
	Between bulls	- 0.07	- 0.25	+ 0.52
	Within bulls	+ 0.15*	+ 0.06	- 0.07
Semen volume	Total	- 0.02	+ 0.09	+ 0.20
	Between bulls	+ 0.02	- 0.08	+ 0.37
	Within bulls	- 0.04	+ 0.15*	+ 0.07
Sperm per ejaculate	Total	+ 0.08	- 0.04	+ 0.22*
	Between bulls	- 0.03	- 0.21	+ 0.49
	Within bulls	+ 0.13	+ 0.03	- 0.04
Initial motility	Total	+ 0.34**	+ 0.32**	+ 0.13
	Between bulls	+ 0.50*	+ 0.55*	+ 0.34
	Within bulls	+ 0.28**	+ 0.25**	+ 0.02
Duration of 2 motility	Total	- 0.03	- 0.01	- 0.08
	Between bulls	+ 0.09	+ 0.26	- 0.32
	Within bulls	- 0.06	- 0.07	+ 0.03
Total duration of motility	Total	- 0.03	- 0.04	- 0.01
	Between bulls	+ 0.01	+ 0.18	- 0.26
	Within bulls	- 0.03	- 0.09	+ 0.08
<i>Diluted semen</i>				
Total duration of motility	Total	+ 0.24**	- 0.05	+ 0.25*
	Between bulls	+ 0.19	0.00	+ 0.25
	Within bulls	+ 0.26**	- 0.07	+ 0.25*
Initial live sperm (%)	Total		+ 0.69**	+ 0.11
	Between bulls		+ 0.80**	+ 0.29
	Within bulls		+ 0.66**	0.00
Live sperm surviving 0° C. (%)	Total			+ 0.04
	Between bulls			+ 0.29
	Within bulls			- 0.17

<sup>a</sup> All coefficients of correlation involving fertility are based on data from 81 semen samples from 14 bulls, while all intra-semen correlations are based on data from 236 semen samples from 19 bulls.

nificance. In addition to the coefficients of correlation listed in the table, additional correlations were determined between the percentage of spermatozoa killed by the 0° C. cold treatment and fertility where the percentage of spermatozoa killed by the cold shock is equal to the difference between the initial per cent live sperm and the per cent following the cold shock. These coefficients of correlation are as follows: "total," + 0.06; "between bull," - 0.13; "within bull," + 0.27\*.

For use in partial and multiple correlations, the following symbols were assigned to certain semen characteristics which had some degree of correlation with fertility: fertility, 1; spermatozoa concentration, 2; initial motility, 3; duration of diluted motility, 4; initial per cent live spermatozoa, 5; and per cent live spermatozoa following 0° C. shock, 6.

Multiple correlations involving fertility calculated on a "total" or "within bull" basis did not raise the zero order coefficients of correlation to any appreciable extent.

TABLE 4

*Zero order and multiple between-bull coefficients of correlation involving fertility and selected semen characters*

	Coefficients of correlation	Degrees of freedom	$r_{0.05}$ or $R_{0.05}$
$r_{12}$	+ 0.52	12	0.53
$r_{13}$	+ 0.34	12	0.53
$r_{14}$	+ 0.25	12	0.53
$r_{15}$	+ 0.29	12	0.53
$r_{16}$	+ 0.29	12	0.53
$R_{1, 23456}$	+ 0.71	8	0.84
$R_{1, 2356}$	+ 0.70	10	0.73
$R_{1, 236}$	+ 0.64	11	0.65
$R_{1, 26}$	+ 0.69*	11	0.65

"Between bull" zero order and multiple coefficients of correlation of interest are presented in table 4. The multiple coefficients of correlation ( $R$ ) involving fertility show that the percentage live spermatozoa following the 0° C. temperature shock and spermatozoa concentration contribute to the only statistically significant multiple correlation ( $R_{1, 26} = + 0.69^*$ ). The further inclusion of semen characteristics 3, 4 and 5 does not materially increase this multiple correlation.

TABLE 5

*Standard partial regression coefficients indicating relative influence of the various independent variables on fertility*

Standard partial regression coefficients			
All variables	Deleting independent variables 3 and 4	Deleting independent variables 3, 4 and 6	Deleting independent variables 3, 4 and 5
$b'_{12, 3456} = + 0.710$	$b'_{12, 26} = + 0.650$	$b'_{12, 5} = + 0.574$	$b'_{12, 6} = + 0.656$
$b'_{13, 2456} = - 0.120$			
$b'_{14, 2356} = - 0.009$			
$b'_{15, 2346} = + 0.154$	$b'_{15, 26} = + 0.126$	$b'_{15, 2} = + 0.372$	
$b'_{16, 2345} = + 0.435$	$b'_{16, 25} = + 0.391$		$b'_{16, 2} = + 0.476$
$R_{1, 23456} = + 0.71$	$R_{1, 256} = + 0.70$	$R_{1, 25} = + 0.64$	$R_{1, 26} = + 0.69^*$

Standard partial regression coefficients also were calculated to help assign relative importances of the independent variables. These are presented in table 5. A consideration of both the multiple correlations and the standard partial regression coefficients indicates that a partial regression equation for predicting the mean fertility levels of bulls ( $\bar{Y}_1$ ) based on the independent vari-

ables, mean spermatozoa concentration ( $X_2$ ) and the mean percentage of live spermatozoa following a 10-min. exposure to a 0° C. temperature ( $X_6$ ), would be most meaningful. This equation is as follows:  $\hat{Y}_1 = 32.40 + 0.0107 X_2 + 0.3248 X_6$ .

Substitution of some mean values for the independent variables in the equation gives the following estimated mean fertility values:

$X_2$	$X_6$	$\hat{Y}_1$
1000	55	61.0
1000	65	64.2
1500	55	66.4
1500	65	69.6

These combinations of values indicate the possible usefulness of this equation for predicting mean fertility levels of bulls based on variations in mean spermatozoa concentrations and mean percentages of spermatozoa in diluted semen surviving a 0° C. cold shock.

#### DISCUSSION

The problem of selecting the semen samples to be used for artificial insemination continues to be a major one. Most studies to date have failed to provide the basis for predicting the fertilizing capacity of a semen sample before it is used in the field. The use of statistical treatment involving "total," "between" and "within bull" correlations, as well as partial and multiple correlations, has not been adequately investigated heretofore, and for that reason these analyses have been included in this study.

In the present study, the correlations of various semen characteristics with fertility obtained on the "total" and "within bull" basis are so low as to have little or no prediction value. This precludes the use of these correlations as a basis for predicting the fertilizing capacity of individual semen samples from bulls in general or from a given bull.

The usefulness of this study then is limited to predictions on a "between bull" basis. Thus, if from a given bull there are a series of semen samples on which determinations for spermatozoa concentration and the percentage of spermatozoa surviving a 0° C. cold shock have been made, the mean values for these two variables could be used in the partial regression equation to predict the relative mean fertility level for this bull. The correlation between actual and predicted mean fertility levels of a group of bulls similar to the ones used in this study should be approximately +0.69\*, according to the multiple coefficient of correlation determined in this study. Thus, approximately 50 per cent of the "between bull" variance in regard to fertility is accounted for by this correlation involving spermatozoa concentration and the percentage of living spermatozoa following a 0° C. cold shock.

#### SUMMARY

The technique of differential staining of live and dead spermatozoa was utilized in this study to determine the relationship of the initial percentage of



live spermatozoa in semen samples, as well as the percentage of spermatozoa in diluted semen surviving a 0° C. temperature for 10 min., to other semen characters and fertility. Data on 236 semen samples from 19 bulls, including 81 samples from 14 bulls on which fertility data were based, were used in this study. An analysis of variance of the data for the various semen characters indicated that highly significant differences existed among the bulls in regard to these semen characters. A correlation analysis was presented on a "total," "between" and "within bull" basis, but only the analysis on a "between bull" basis had coefficients of correlation sufficiently high to have any prediction value. A multiple coefficient of correlation of +0.69\* of fertility with spermatozoa concentration and the percentage of spermatozoa surviving a 0° C. cold shock was obtained. A partial regression equation involving these factors was presented.

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# DIFFERENCES IN MILK AND BUTTERFAT PRODUCTION AND TEST OF Ayrshire COW FAMILIES<sup>1</sup>

KENNETH A. TABLER,<sup>2</sup> W. J. TYLER AND GEORGE HYATT, JR.

*West Virginia University, Morgantown*

The selection of dairy cattle for production may be based on the records of the individual, of the ancestors and other close relatives and of the progeny. The method where the records of the individual alone are considered is referred to as individual selection. On the other hand, the breeder practicing family selection considers the average of the family to which individuals belong and only individuals from the best families are saved, regardless of their individual merit.

Cow families which are considered to be outstanding in production or butterfat test have been discussed frequently in dairy magazines and breed journals. Bartlett and Margolin (1) noted that specific cow families and sires have the ability consistently to transmit genetic factors for superior size, butterfat percentage, milk production and total butterfat.

The object of this study was to compare a number of cow families to determine if there were significant differences between them in milk production, butterfat test and butterfat production. In addition, three methods of selection on the basis of cow families were compared with individual selection as a means of improving the average production of a herd.

The cow families in this study consisted of descendants of individuals which were the foundation animals at the time the herd was started. This is a common definition of cow families in use today, and is the one that is suggested most frequently for cow family analysis work.

## EXPERIMENTAL PROCEDURE

The data consisted of records collected between 1922 and 1948 in the Reymann Memorial Herd of Ayrshires owned by the West Virginia Agricultural Experiment Station. The individual milk production, butterfat production and butterfat test records of 401 Ayrshire females were studied. These cows were progeny of 46 different sires and were members of 19 different cow families.

During the period covered, all milking cows were fed a home-mixed grain ration containing approximately 12 per cent digestible crude protein. The grain fed was limited to 14 lb. per day for cows and 10 lb. per day for first-calf heifers. The animals also were fed a good quality hay, silage when available and pasture in season. In addition, as far as possible, heifers were bred to freshen in the

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<sup>2</sup> Assistant in Dairy Science at the University of Illinois, Urbana.

months of September, October and November, which helped to standardize feeding and management conditions.

The first available lactation record was used for each animal so that selection of records was not involved in this study. The lactation records were standardized to a 305-day, twice-a-day milking, mature-equivalent basis. In addition, the milk records were adjusted to a 4 per cent milk basis by the use of Gaines' (2) formula.

The cows in each of the 19 families were divided on the basis of their sire into subgroups. For example, all the cows in the *N* family that were sired by number 19 were in one subgroup. For the 19 cow families, the 401 cows in the production study made up 269 different sire-family subgroups. Both families and subgroups were analyzed by the analysis of variance (4) to test the significance of differences between families, between sires and between families within sires.

TABLE 1

*The number of cows and their average milk (M.E. 4%) production, butterfat test and butterfat (M.E.) production for 19 cow families in the Reymann Memorial Herd from 1922-1948*

Family	No. of cows	Milk M.E. 4%	Butterfat test	Butterfat M.E.
		(lb.)	(%)	(lb.)
A	31	9,748	4.30	402
B	17	9,596	4.34	383
C	29	9,388	4.31	387
D	22	9,144	4.27	375
E	14	9,127	4.16	371
F	35	8,873	4.30	364
G	45	8,828	4.35	365
H	18	8,786	4.16	356
I	12	8,729	4.33	360
J	24	8,634	4.11	350
K	25	8,498	4.18	346
L	13	8,454	4.26	346
M	19	8,454	4.17	343
N	10	8,381	4.44	350
O	13	8,068	4.32	334
P	37	7,988	4.24	326
Q	14	7,747	4.17	319
R	12	7,703	4.45	321
S	11	7,543	4.10	305

## RESULTS

The milk (M.E., 4 per cent, 2 ×, 305 day) production, butterfat test and butterfat (M.E., 2 ×, 305 day) production averages for the 19 cow families in the Reymann Memorial Herd are given in table 1. Each family has ten or more cows in it and is represented by at least one member in the herd in 1947. There were other cow families which no longer exist. The milk production ranged from 7,543 lb. in the *S* family to 9,748 lb. in the *A* family. The butterfat test ranged from 4.10 in the *S* family to 4.45 in the *R* family, while the butterfat production ranged from 305 lb. in the *S* family to 402 lb. in the *A* family.

The daughter averages for the 46 sires of the 401 cows ranged from 5,903 to 14,417 lb. of milk, from 238 to 604 lb. of butterfat and from 3.90 to 4.73 per cent of butterfat.

The first production records of the 401 cows in the 19 cow families were analyzed by the analysis of variance and the results are shown in tables 2, 3 and

TABLE 2

*Analysis of variance of M. E. milk on 401 cows in the Reymann Memorial Herd*

Source of variation	Degrees of freedom	Mean square	Per cent of total variance
Between families	18	7,638,888**	
Within families	382	3,647,736	
Between subgroups	268	4,655,839	
Between sires	45	14,791,738**	36
Between families within sires	223	2,610,479	8
Within subgroups	132	2,145,229	56
Total	400	3,827,338	100

\*\*  $P < 0.01$  (Highly significant).

4 In these tables the variation is divided into (a) variation due to differences between families, (b) variation due to differences within families, (c) variation due to differences between subgroups (cows in the same family and by the same sire) and (d) variation due to differences within subgroups, which is a measure of the average variation between records of cows in each of the subgroups. Variation due to c is further divided into differences between sires and differences between families within sires.

TABLE 3

*Analysis of variance of butterfat test on 401 cows in the Reymann Memorial Herd*

Source of variation	Degrees of freedom	Mean square	Per cent of total variance
Between families	18	0.169**	
Within families	382	0.091	
Between subgroups	268	0.106	
Between sires	45	0.220**	17
Between families within sires	223	0.083	8
Within subgroups	132	0.071	75
Total	400	0.094	100

\*\*  $P < 0.01$  (Highly significant).

The differences between families and also differences between sires were highly significant for milk production and butterfat test and were significant for the butterfat production. Part of the variation between families in milk production, butterfat test and butterfat production may be attributed to the fact that some sires were used more heavily on some families than on others.

Sire differences accounted for 36 per cent of the variation in milk and fat records and for 17 per cent of the variation in test. The differences between sires, however, are not caused entirely by variation in the breeding values of the sires, but also are influenced by environmental variations. The nature of the experiment does not permit a direct division between sire and year-to-year effect. Although every attempt was made to keep herd environment constant from year to year, it is highly probable that conditions that affect milk and fat production did change from year to year. In addition, it is possible that culling during the 26-yr. period aided in differentiating between families. However, this source of variation would be taken out in the between-sire portion of the variance. This may explain to some extent why sires appear to have been so important in distinguishing between families. The within-sire variation between families for milk production and test do give some indication, however, that some differentiation between families may have occurred. In tables 2 and 3,

TABLE 4  
*Analysis of variance of M. E. butterfat on 401 cows in the Reymann Memorial Herd*

Source of variation	Degrees of freedom	Mean square	Per cent of total variance
Between families	18	12,891*	
Within families	382	7,310	
Between subgroups	268	8,860	
Between sires	45	29,228**	36
Between families within sires	223	4,749	
Within subgroups	132	4,926	64
Total	400	7,562	100

\*  $P < 0.05 > 0.01$  (Significant).

\*\*  $P < 0.01$  (Highly significant).

8 per cent of the total variation for both milk production and percentage of butterfat could be ascribed to differences between families within sires. However, the number of cattle in this study is too few to establish statistical significance for this source of variation.

*Genetic relationship.* In studying differences between families, it is important to know the genetic relationship of members of the same family. The higher the genetic relationship between members of the same family, the more the genetic variation will be between rather than within families. Also, the higher the genetic relationship, the more effective selection between families will be. The average genetic relationship (5) of members of the same family was computed to be 15 per cent. Since half-sisters, either paternal or maternal, and daughters and granddams are 25 per cent related, this relationship between members of the same family is not very high. In general, the genetic relationship decreases as the number of cows in the same family increases. However, the more heifer calves a particular cow has, the closer the family structure is woven, thus increasing the average genetic relationship.

*Selection study.* It was possible to make a comparison of a few theoretical selection methods because all normal females dropped in the Reymann Memorial Herd must complete their first lactation record before they can be culled. Therefore, at least one unselected record was available on all cows that freshened. These theoretical comparisons of different methods of selection were based on the first mature equivalent (M.E.) 4 per cent milk record of animals in the cow families. Individual selection was compared with three different methods of family selection. For these comparisons, selection arbitrarily was started in 1928 and comparisons were made for the two successive 10-yr. periods, 1928 to 1937 and 1938 to 1947. It was possible to obtain an idea of the outstanding families in 1928 by using the average production for each family between 1922 and 1927.

On the individual basis, two-thirds of the highest producing cows on the basis of their first lactation performance would have been selected as breeding stock each year, beginning in 1928, and the remaining one-third would have been culled. Once a cow was placed in either one or the other of the groups, she remained in this group until she left the herd, regardless of her future performance. If a cow was placed in the cull group, in practice, she would have been culled from the herd. Therefore, if she had any daughters in this herd after she was placed in the cull group, they automatically were placed in the group of culls and when they freshened, they were not considered in the division of the first calf heifers into the highest two-thirds and lowest one-third groups.

The three methods of family selection that were used are listed in table 5. The average milk records for the selected individuals and their descendants also are given in table 5. Individual selection would have increased the herd average from 7,632 lb. of milk (the 1922 to 1927 herd average) to 10,434 lb. of milk for the 1938 to 1947 herd average, or an increase of 2,802 lb. of milk. The offspring of the culls between 1938 and 1947 would have averaged 662 lb. of milk more than the 1922 to 1927 average of 7,632 lb.

Selection for milk production from the twelve highest-average producing families where only two-thirds of the highest cows in each family were kept with the lower one-third of the cows in each of these twelve families and the seven lowest-averaging families being culled, was the best method of family selection and would have increased the 1938 to 1947 herd average over the 1922 to 1927 herd average by 2,280 lb. of milk (9,912 minus 7,632). The offspring of the culls during the same period averaged 1,556 lb. of milk more than the 1922 to 1927 average.

If the twelve families with the highest average production had been selected and the seven families with the lowest average production had been culled, the 1938 to 1947 herd average would have been increased an average of 2,135 lb. of milk over the 1922 to 1927 average (9,767 minus 7,632). The offspring of the group of culls between 1938 and 1947 would have produced 1,333 lb. of milk more than the 1922 to 1927 average.

Where the third method of family selection was used, the 1938 to 1947 herd average was increased by 2,021 lb. of milk over the 1922 to 1927 average (9,653

minus 7,632). On the other hand, the progeny of the culls during the same period produced 1,768 lb. of milk more than the 1922 to 1927 average.

#### DISCUSSION

The differences between families for milk production, butterfat test and butterfat production all were significant. Part of this difference could be attributed to the fact that some sires were used more heavily on some families than they were on others. When the sire differences were eliminated, the remaining variations between families were not statistically significant. Thus, the sires contributed substantially to the differences between families—at least enough to

TABLE 5

*Theoretical comparisons of selection methods based on the first M. E. 4 per cent milk record of animals in the Reymann Memorial Herd*

Basis of selection	Average M. E. 4% milk production					
	1922-1927		1928-1937		1938-1947	
	No.	Av.	No.	Av.	No.	Av.
		(lb.)		(lb.)		(lb.)
Individual (on the yearly basis):						
$\frac{1}{3}$ highest producers	64	7,632	75	8,625	117	10,434
$\frac{1}{3}$ lowest producers (culls)			54	6,515	78	8,294
12 families with the highest average production between 1922 and 1927	44	8,121	105	7,704	149	9,767
7 families with the lowest average production between 1922 and 1927 (culls)	20	6,557	24	7,905	46	8,965
Highest $\frac{1}{3}$ of the cows in each of 12 highest averaging families between 1922 and 1927	29	9,011	68	8,617	105	9,912
Lowest $\frac{1}{3}$ of the cows in each of 12 highest averaging families, plus all members of 7 lowest averaging families between 1922 and 1927 (culls)	35	6,490	61	6,765	90	9,188
Highest $\frac{1}{3}$ of the cows in each of 12 highest averaging families, plus highest $\frac{1}{3}$ of the cows in each of 7 lowest producing families between 1922 and 1927	36	8,825	77	8,676	137	9,653
Lowest $\frac{1}{3}$ of the cows in each of 12 highest averaging families, plus lowest $\frac{1}{3}$ of the cows in each of 7 lowest producing families between 1922 and 1927 (culls)	28	6,098	52	6,358	58	9,400

make the differences significant. Although the differences between families within sires, which is a measure of the remaining differences between families after the sire differences are removed, are not significant, there are considerable differences left between families that may be important.

While the genetic relationships of members of the same cow family were rather low, this is somewhat offset by the fact that the environment was approximately the same for each family. Therefore, selection for differences between families in the same herd should be more effective than selection between families in different herds because differences between families in the same herd are

mostly genetic, if the environment is nearly random with respect to families (3). Thus, the environmental portion of records should be taken into consideration when differences between families kept in different herds are studied or compared.

The results of the selection study showed that individual selection for milk production was superior to family selection. Family selection where all members of a family were retained was not as effective in increasing milk production as when some individual selection was practiced within each family. Family selection would be expected to be superior to individual selection, if the phenotypic or observed correlation between members of the same family was very low and the genetic relationship of members of the same family was very high. The phenotypic correlation in these data for milk production was 0.05, for butterfat test, 0.04, and for butterfat production, 0.035. The average genetic relationship of these families was about 15 per cent. Lush (3) has developed a formula for comparing the relative effectiveness of family and individual selection. When the values for milk production were substituted into Lush's formula,<sup>3</sup> the results indicated that family selection could be expected to be 61.5 per cent as effective as individual selection in increasing the average milk production of the herd. The genetic relationship between members of the cow families in this herd would have to be about 28 per cent now for family selection to be equal to individual selection.

The genetic improvement in the producing ability of the Reymann Memorial Ayrshire cow families was due, in part, to the proved sires that were used on these cow families. The artificial insemination programs now make it possible for most dairymen to improve the producing abilities of their cattle by using the service of well-proved sires. Through this system of breeding, cow families will improve as well as the general level of production of herds in which the proved bulls are used.

#### SUMMARY

The milk production, butterfat test and butterfat production records of 401 animals that were members of 19 cow families were analyzed by the analysis of variance. The results showed that there were significant differences between the cow families in the three production figures studied. Most of these variations could be attributed to sire differences between families. When the sire differences were removed, the remaining variations between families were too small to be statistically significant. The differences between sires for the production figures were highly significant. Yearly variations contributed to the differences between sires. The average genetic relationship between members of the same cow family was 15 per cent.

Three methods of selection of breeding females on the basis of family su-

$$s = \frac{1 + (n-1)r}{\sqrt{n[1 + (n-1)t]}}$$

$n$  = number of individuals in each family.

$r$  = genetic relationship between members of the same family.

$t$  = phenotypic correlation between members of the same family.



periority in milk production were compared with selection on individual performance as a means of improving the production of the herd. Individual selection for milk production was superior to selecting females on a family basis. Family selections appeared to be more effective where some individual selection was practiced within the family than where all members of the best families were retained for breeding purposes.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. V. YIELD,  
SPECIFIC GRAVITY AND CONCENTRATIONS OF TOTAL SOLIDS  
AND ITS VARIOUS COMPONENTS OF COLOSTRUM  
AND EARLY MILK<sup>1</sup>

D. B. PARRISH, G. H. WISE,<sup>2</sup> J. S. HUGHES AND F. W. ATKESON

*Kansas Agricultural Experiment Station, Manhattan*

Considerable information on specific gravity and on concentrations of total solids, fat, protein, lactose and ash of colostrum has been accumulated. From the early studies, many of which are referred to by Houdinière (7), Overman and Sanmann (8) and Weber (18), there emerged a general picture of the gross composition of colostrum and a recognition of its variability. Within the past 25 yr., several studies of changes in the principal constituents of the mammary secretions during the transition period have been reported (3, 4, 5, 8, 12, 13, 15, 16, 17); however, none of these investigations included more than 15 animals. Van der Burg (19) of Holland published data on the composition of 163 colostrum samples collected over a period of 12 yr., but only the first postpartum milkings are included in his report. Hills (6) apparently was the first in this country to report results of colostrum studies.

In view of the limited amount of data on yields, specific gravity and concentrations of the major components in colostrum and early milk from breeds of dairy cattle common to this country, further study of the foregoing factors was considered desirable.

EXPERIMENTAL PROCEDURES

*Feeding and management of cows.* Samples of colostrum and early milk were obtained from 111 cows of four breeds, Holstein, Ayrshire, Jersey and Guernsey. These animals, all free of gross abnormalities, represented approximately two-thirds of the cows that calved in the College herd during the 3 yr. of the study. Cows that previously had lactated were given a conditioning period of 4 to 8 wk. before parturition.

The typical herd ration fed to most of the cows consisted of a concentrate mixture, Atlas sorgo silage and hay. Pasture grazing was allowed whenever it was available. Because of a period of grain scarcity, several cows received pasture as the principal feed prepartally, concentrates being fed only postpartally. Some cows received a winter barn ration that deviated from normal in that the levels of proteins in the concentrate mixture were either more or less than 16 per cent (9); others were supplemented with vitamin A and/or tocopherol concentrates (10, 11).

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<sup>1</sup> Contribution no. 405, Department of Chemistry and no. 192, Department of Dairy Husbandry.

<sup>2</sup> Present address: Department of Animal Industry, North Carolina State College, Raleigh.

*Sampling procedure.* In order that representative samples of the mammary secretions be obtained, calves were not allowed to nurse and the udder was evacuated as completely as possible by either hand or machine milking twice daily. The total collection at each milking during the first 14 days was weighed, and those selected for analyses were mixed and sampled carefully. If samples could not be analyzed immediately, they were stored at 4° C. for periods not to exceed 5 days. All samples were adjusted to a temperature of 20° C. and mixed before portions for analyses were removed.

*Analyses made:* (a) *Specific gravity.* This property was determined at 20° C. using a Mohr's balance. Though this procedure is considered to be less accurate than that employing a pycnometer, use of the latter is time-consuming. Values obtained in a series of tests of viscous colostrum using a pycnometer were either the same as or not more than 0.004 lower than those obtained by use of the balance.

TABLE 1  
*Results from different procedures of analyzing colostrum for fat content*

Sample no.	Per cent fat		
	Babcock method		Mojonnier method
	Volumetric sampling	Gravimetric sampling	
C-1	1.65	1.60	1.79
C-2	2.90	2.80	2.86
C-3	3.85	3.70	3.88
C-4	2.90	2.80	2.81
C-5	7.35	7.25	7.28
C-6		11.20	11.10
C-7	7.10	7.00	7.06

(b) *Total solids.* Approximately 3 ml. of the mammary product were weighed into a dish containing fine dry sand and heated at 100° C. overnight. The use of sand in the dishes was necessary to obtain reproducible results on replicate samples of viscous colostrum.

(c) *Fat.* Percentage of fat was determined by the Babcock method for whole milk. Comparisons of results on a series of samples using the Babcock and the Mojonnier procedures are shown in table 1. Although the methods yielded slightly different results on individual samples, the differences were so small that the more rapid and less laborious Babcock procedure was adopted.

(d) *Solids-not-fat.* This value was obtained by subtraction of percentage of fat from that of total solids.

(e) *Total protein.* Total protein values reported herein were obtained by subtraction of the sum of percentages of fat, lactose and ash from the percentage of total solids. For comparative purposes, protein in samples from twenty cows was determined by Rowland's (14) semi-micro Kjeldahl procedure and also by difference (table 2). By the latter method, average percentage protein was consistently higher than by the former, but only from 0.07 to 0.95 per cent. Thus,

calculations by difference seem to be adequate for studying the marked changes occurring in proteins of the secretions during the transition to normal milk.

(f) *Lactose*. The polarimetric A.O.A.C. method for lactose (1) was modified to the extent that the first 10 to 15 ml. of filtrate were discarded in order to obtain solutions suitable for polarization. In the case of samples of a high solids content, only a "one-*N* weight" of sample was used. Overman and Sanmann (8) also found the smaller samples sometimes advantageous.

(g) *Ash*. Ash was determined by evaporating 8 to 10 g. of sample to dryness in a porcelain dish and heating overnight at a temperature of 550° C.

## RESULTS

*Yields of colostrum and early milk*. Average yields by groups of cows of four dairy breeds are shown in fig. 1. Except for a deviation at the second milking for Guernseys, the rates of change in production during the first 2 wk. were similar for all breeds. Quantities obtained at the first milking varied from 3.1 lb. (from a first-lactation Jersey) to 61.8 lb. (from a second-lactation Holstein). On a percentage basis, variations in yields within breeds were large in the first post-

TABLE 2

*Comparisons of methods of calculation of concentrations of total protein in colostrum and early milk from 20 cows*

Method of calculation	Per cent protein in milking no.								
	1	2	3	4	5	6	7 + 8 <sup>a</sup>	15 + 16	27 + 28
Nitrogen × 6.38	16.46	10.30	5.88	4.59	4.16	4.12	3.96	3.48	3.19
By difference	17.41	10.93	6.38	4.68	4.32	4.28	4.12	3.61	3.26

<sup>a</sup> Sample composited on basis of yields.

partum milkings but decreased as total production increased. Average production during the first 2 wk. was highest by Holsteins and lowest by Jerseys, whereas that by Ayrshires and by Guernseys was similar but somewhat greater than that by Jerseys and less than that by Holsteins.

*Specific gravity*. Specific gravity of the early mammary secretions decreased rapidly in successive milkings, most of the change being completed before the fourth milking (table 3). Specific gravity of the secretions continued to decrease after the fourth milking, but at a less rapid rate.<sup>1</sup>

Lowest specific gravity values usually were found in secretions from Holsteins; however, marked variations were found within each breed. Four Jerseys (two of them first-lactation animals) and one Ayrshire produced first-milking colostrum having a specific gravity above 1.080. First-milking secretions from three other Jerseys, also first-lactation animals, had specific gravities between 1.027 and 1.034. In these latter secretions, concentrations of fat and of total solids were low. These properties, however, were normal by the end of the usual transi-

<sup>1</sup> Statements in this report concerning rates of change are based on graphic analysis (semi-log paper), which are not shown in order to conserve space.

TABLE 3

*Specific gravity and concentrations of various constituents in colostrum and early milk*

Breed	No. of milking							
	1	2	3	4	5+6 <sup>a</sup>	7+8	15+16	27+28
Specific gravity								
Holstein . . .	1.056 (29) <sup>b</sup> ±0.01110 <sup>c</sup>	1.040 (29) ±0.00536	1.035 (29) ±0.00287	1.033 (29) ±0.00193	1.033 (29) ±0.00130	1.033 (27) ±0.00119	1.032 (25) ±0.00114	1.032 (5) ±0.00187
Ayrshire . . . .	1.064 (31) ±0.01040	1.048 (31) ±0.00853	1.038 (31) ±0.00593	1.035 (29) ±0.00332	1.034 (31) ±0.00217	1.034 (30) ±0.00191	1.032 (29) ±0.00170	1.032 (13) ±0.00210
Jersey . . . . .	1.063 (32) ±0.01439	1.049 (32) ±0.01131	1.040 (32) ±0.00580	1.036 (32) ±0.00283	1.035 (31) ±0.00152	1.035 (29) ±0.00133	1.034 (26) ±0.00147	1.033 (6) ±0.00141
Guernsey . . . . .	1.065 (19) ±0.00776	1.045 (19) ±0.00683	1.037 (19) ±0.00383	1.035 (19) ±0.00325	1.035 (19) ±0.00141	1.034 (19) ±0.00187	1.033 (18) ±0.00129	1.031 (4) ±0.00173
Solids (%)								
Holstein . . .	23.9 (10) ±3.408	17.9 (10) ±2.042	14.1 (10) ±1.275	13.9 (10) ±0.923	13.6 (10) ±0.641	13.7 (9) ±0.975	13.6 (10) ±0.784	12.9 (5) ±0.687
Ayrshire . . .	24.2 (16) ±3.803	21.8 (16) ±4.402	16.3 (16) ±2.332	15.1 (15) ±1.702	15.4 (16) ±2.049	15.3 (15) ±2.157	14.8 (16) ±1.692	14.0 (12) ±1.016
Jersey . . . . .	22.5 (16) ±6.741	19.1 (16) ±5.769	15.3 (16) ±2.786	14.2 (16) ±1.374	14.4 (16) ±0.919	14.4 (16) ±0.971	14.3 (14) ±1.055	14.3 (6) ±0.574
Guernsey <sup>d</sup> . . .	30.4 (4) ±1.229	22.8 (4) ±4.093	16.1 (4) ±1.133	14.9 (4) ±1.127	14.1 (4) ±1.253	14.7 (4) ±1.308	14.3 (4) ±1.207	14.4 (4) ±0.854
Fat (%)								
Holstein . . . .	6.7 (29) ±2.650	5.4 (29) ±1.683	3.9 (29) ±1.038	4.4 (29) ±1.242	4.3 (29) ±0.724	4.4 (27) ±1.076	4.3 (25) ±0.787	4.0 (5) ±0.541
Ayrshire . . . .	5.1 (31) ±2.040	5.8 (31) ±2.306	5.0 (31) ±1.576	4.9 (29) ±1.290	4.9 (31) ±1.441	5.2 (30) ±1.736	5.2 (29) ±1.270	4.9 (12) ±0.891
Jersey . . . . .	4.2 (32) ±1.812	5.0 (32) ±2.697	4.3 (32) ±1.539	4.3 (32) ±0.995	4.4 (31) ±0.858	4.7 (29) ±1.340	5.0 (26) ±1.153	5.0 (6) ±0.427
Guernsey . . . .	6.8 (19) ±1.780	6.1 (19) ±1.768	5.7 (19) ±1.233	4.7 (19) ±1.258	4.6 (19) ±1.296	5.0 (19) ±1.272	5.0 (18) ±0.821	5.2 (4) ±0.806

	16.7 (10)	12.2 (10)	9.8 (10)	9.4 (10)	9.5 (10)	9.3 (9)	9.1 (10)	8.8 (5)
Holstein	$\pm 3.029$	$\pm 1.566$	$\pm 2.812$	$\pm 1.619$	$\pm 0.402$	$\pm 0.200$	$\pm 0.321$	$\pm 0.371$
Ayrshire	19.1 (16)	14.4 (16)	11.1 (16)	10.1 (15)	9.9 (16)	9.8 (15)	9.4 (16)	9.1 (12)
	$\pm 3.469$	$\pm 2.399$	$\pm 1.445$	$\pm 1.202$	$\pm 0.774$	$\pm 0.442$	$\pm 0.379$	$\pm 0.464$
Jersey	18.2 (16)	15.0 (16)	11.2 (16)	10.3 (16)	10.0 (16)	10.0 (16)	9.7 (14)	9.4 (6)
	$\pm 5.574$	$\pm 4.586$	$\pm 2.259$	$\pm 1.076$	$\pm 0.352$	$\pm 0.274$	$\pm 0.325$	$\pm 0.467$
Guernsey <sup>d</sup>	23.0 (4)	16.2 (4)	11.1 (4)	9.8 (4)	9.7 (1)	9.8 (4)	9.4 (4)	9.2 (4)
	$\pm 0.742$	$\pm 2.918$	$\pm 0.922$	$\pm 0.153$	$\pm 0.252$	$\pm 0.234$	$\pm 0.252$	$\pm 0.163$

Total protein (%)								
Holstein	14.0 (8)	8.4 (8)	5.1 (8)	4.2 (8)	4.1 (8)	3.9 (7)	3.4 (8)	3.1 (6)
	$\pm 2.585$	$\pm 3.133$	$\pm 1.680$	$\pm 0.748$	$\pm 0.526$	$\pm 0.328$	$\pm 0.260$	$\pm 0.325$
Ayrshire	15.7 (9)	9.9 (9)	5.6 (9)	4.6 (9)	4.1 (9)	4.2 (8)	3.5 (8)	3.1 (6)
	$\pm 1.542$	$\pm 3.289$	$\pm 1.459$	$\pm 0.751$	$\pm 0.423$	$\pm 0.431$	$\pm 0.360$	$\pm 0.356$
Jersey	14.2 (11)	9.9 (11)	5.8 (11)	4.7 (11)	4.4 (11)	4.2 (11)	3.9 (10)	3.8 (4)
	$\pm 5.257$	$\pm 4.510$	$\pm 2.510$	$\pm 1.090$	$\pm 0.501$	$\pm 0.333$	$\pm 0.117$	$\pm 0.784$
Guernsey <sup>d</sup>	20.0 (4)	11.9 (4)	6.1 (4)	4.4 (4)	4.2 (4)	4.2 (4)	3.7 (4)	3.3 (4)
	$\pm 0.666$	$\pm 3.380$	$\pm 1.058$	$\pm 0.318$	$\pm 0.065$	$\pm 0.122$	$\pm 0.238$	$\pm 0.548$

Lactose (%)								
Holstein	2.7 (8)	3.9 (8)	4.4 (8)	4.6 (8)	4.7 (5)	4.8 (7)	4.9 (8)	5.0 (6)
	$\pm 0.911$	$\pm 0.451$	$\pm 0.208$	$\pm 0.268$	$\pm 0.212$	$\pm 0.192$	$\pm 0.100$	$\pm 0.112$
Ayrshire	2.6 (9)	3.4 (9)	4.2 (9)	4.4 (9)	4.7 (9)	4.8 (8)	4.9 (9)	5.1 (6)
	$\pm 0.605$	$\pm 0.737$	$\pm 0.469$	$\pm 0.337$	$\pm 0.362$	$\pm 0.234$	$\pm 0.212$	$\pm 0.037$
Jersey	2.4 (11)	3.3 (11)	4.3 (11)	4.7 (11)	4.9 (11)	5.0 (11)	5.0 (10)	4.9 (4)
	$\pm 0.771$	$\pm 0.911$	$\pm 0.697$	$\pm 0.346$	$\pm 0.226$	$\pm 0.161$	$\pm 0.182$	$\pm 0.277$
Guernsey <sup>d</sup>	1.8 (4)	3.3 (4)	4.2 (4)	4.6 (4)	4.7 (4)	4.8 (4)	4.9 (4)	5.1 (4)
	$\pm 0.970$	$\pm 0.535$	$\pm 0.277$	$\pm 0.342$	$\pm 0.191$	$\pm 0.208$	$\pm 0.082$	$\pm 0.100$

		%
Holstein	95	.74
	.081	$\pm 0.031$
Ayrshire	.07	.77
	.182	$\pm 0.047$
	.06	.83
	.149	$\pm 0.039$
		.82
		$\pm 0.026$

tion period. Colostrum having specific gravities of 1.025 and 1.028 was obtained from two second-lactation Holsteins. Part of the explanation for these low values was the fat content, 13 and 18 per cent, respectively. Several first-milking colostrum samples had specific gravities above 1.060 and fat content greater than 7 per cent, but frequently high fat values seemed to be associated with specific gravities lower than would be expected from concentrations of total solids.

*Total solids.* Solids decreased rapidly in the colostrum secretions during the transition period (table 3). Rates of change were similar to those observed in specific gravity. Except in the first sample of colostrum, Holsteins produced secretions of the lowest content of solids. Content of solids in first-milking colostrum from the Guernseys was appreciably higher than that from the other breeds. Solids of first-milkings varied from 9 to 33.9 per cent.

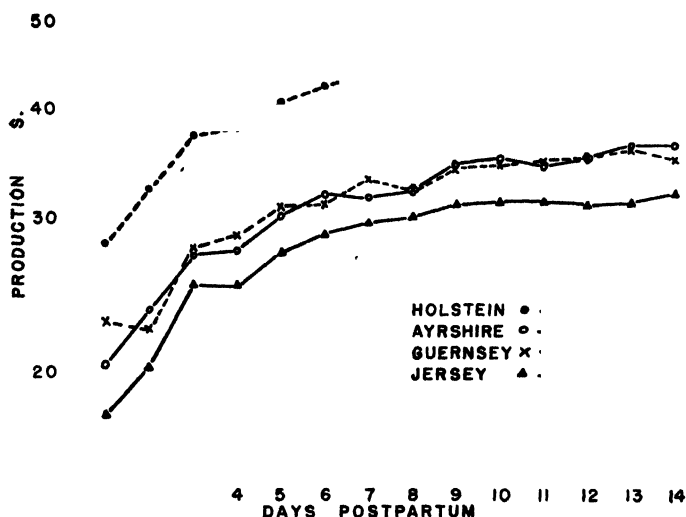


FIG. 1. Average daily production of colostrum and early milk by different breeds of cows. Samples were collected from 29 Holsteins, 31 Ayrshires, 32 Jerseys and 19 Guernseys.

*Fat.* Fat content of early mammary secretions was variable, not only among the different breeds (table 3) but also among individual cows of the same breed. The range in values of fat content of first-milking colostrum was from 0.3 per cent in a sample from a first-lactation Ayrshire to 12.5 to 18.0 per cent in samples from four Holsteins in first to third lactations.

*Solids-not-fat.* The principal difference found when data for solids-not-fat (table 3) and for total solids were plotted and compared was that smoother curves were obtained for the former. The variability of the fat content accounts for the difference. For the first three milkings, the solids-not-fat data from each breed closely followed a logarithmic decrease.

*Total protein.* Trends of changes in levels of proteins (table 3) were similar to those found for specific gravity and for total solids, the decreases following approximately a logarithmic path through the fourth milking. From this stage until the end of the period studied, further reduction occurred, but it was at a sharply reduced rate. Most of the decline in total proteins is traced to the reduction of the globulin content (9). The range of values for total proteins of first-milking colostrum was from 4.0 to 24.6 per cent; these extremes were obtained from first-lactation Jerseys.

*Lactose.* This constituent (table 3) changed inversely with specific gravity, solids, proteins and ash during the transition period. Lactose of first colostrum from Holsteins was highest and that from Guernseys was lowest, but differences in samples from the four breeds became smaller as the secretions changed from colostrum to normal milk. A sample of colostrum from a first-lactation Guernsey analyzed only 0.4 per cent lactose, which was the lowest value found; the highest was 4.1 per cent, in a sample from a first-lactation Holstein.

*Ash.* Changes in ash content of the mammary secretions (table 3) during the early part of the transition were not so marked as were those of specific gravity, solids and proteins. Extremes in ash concentrations of first colostrum were 0.81 and 1.47 per cent, in samples from a Holstein and a Jersey, respectively.

#### DISCUSSION

In all properties and constituents studied, early colostrum from different individuals was considerably more variable than was milk after the transition had occurred. Similar observations were made on proteins (9) and on vitamin A and carotenoids (10). Data in the literature, previously cited, also point to variation as a general characteristic of colostrum. General trends in composition of the secretions during the transition are in agreement with most of the earlier observations reported.

Fat was the most variable constituent studied. In consecutive samples from a number of animals, marked differences were found in concentrations of this component. Examination of production data along with those on fat content indicated that, in a number of cases, these variations might have been the result of incomplete milking. Use of oxytocin to stimulate let-down of milk (2) might have decreased the degree of variation in fat content noted.

In order to identify additional factors that caused variability in quantities and composition of the mammary secretions from different animals, the data (not shown) were arranged by breed, lactation number and season. During the early part of the transition, the average yields of mammary secretions were consistently lower for first-lactation cows than for those previously lactating. There was a tendency for production to be higher during the pasture-grazing season. In many cases solids were a little higher in secretions from first-lactation cows than from those previously lactating. Specific gravity, solids, fat and ash seemed to average slightly higher in samples collected during the winter barn-feeding period than in samples collected at other times. Other differences dependent upon number of previous lactations, season and/or type of ration were not indi-



cated by the data. Furthermore, variations in properties of secretions from animals of the same groups were so marked that the aforementioned differences should be regarded only as possible trends. Further studies are indicated before definite conclusions are justified.

In most cases, colostrum contained more nutrients than did milk, as a result of a higher concentration of proteins, minerals and sometimes fat. Colostrum from the Holsteins and the Guernseys had an energy content<sup>4</sup> about twice that of normal milk, a ratio similar to that calculated by Houdinière (7); but colostrum from Ayrshires and from Jerseys had an energy content only about 1.5 times that of the later milk. However, based on equal weights of dry matter, milk had an energy value either the same as or higher than first-milking colostrum. In agreement with an observation of Engel and Schlag (3), normal milk had a higher ratio of ash to total solids than did first-milking colostrum.

On an energy basis, proteins contributed two to three times more to the total nutritive value of colostrum than to that of normal milk. On the other hand, lactose in milk contributed 2.5 to 5 times as much to the total energy as it did in colostrum, whereas fat in milk constituted 10 to 50 per cent more of the total energy than it did in colostrum.

#### SUMMARY

A study was made of changes in yields, specific gravity, total solids, fat, solids-not-fat, total proteins, lactose and ash in mammary secretions collected from dairy cows during the transition period from colostrum to normal milk.

Considerable variation was found in values for yield and for the properties of early mammary secretions collected from different individuals at the same postpartal period. Fat was the most variable constituent. Variability in secretions from different individuals decreased as transition to normal milk progressed.

Yields of mammary secretions increased markedly during the first week postpartum.

Specific gravity, total solids, solids-not-fat, total proteins and ash decreased rapidly during the first four to six milkings, but only relatively small decreases were noted throughout the remainder of the 14 days of the study. Lactose changed in approximately an inverse ratio to the aforementioned properties.

Based on equal weights of dry matter, milk had an energy value the same as or higher than that of colostrum. Proteins contributed a greater percentage to the total energy value of colostrum than to that of milk, while lactose and fat contributed a greater percentage of energy to milk than to first colostrum. The ratio of ash to total solids was higher in milk than in colostrum.

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<sup>4</sup> Calculated from data of table 3 and the simple formula, relative energy value = protein + lactose + fat  $\times$  2.25.

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# EFFECT OF INCUBATION TEMPERATURES ON THE RETENTION OF BACTERIOPHAGE BY A CULTURE OF *STREPTOCOCCUS LACTIS*<sup>1</sup>

H. F. FORD AND F. J. BABEL

*Dairy Department, Purdue University, Lafayette, Indiana*

Mother cultures propagated in a dairy or cheese plant frequently fail to produce acid at a normal rate, as evidenced by the time required for the culture to coagulate milk. The duration of slow acid production by mother cultures is quite variable and may last for a few days or weeks, or the culture may never produce acid at its previous rate. When a mother culture fails to produce acid satisfactorily, it generally is discarded and a new culture obtained. However, the policy of some plants is to hold a slow acid-producing culture at a favorable growth temperature until coagulation occurs and then make further transfers from it. Future transfers from such a culture may show normal acid production after a few transfers. Cultures obtained from cheese plants and dairies experiencing difficulty with slow mother cultures for periods of rather short duration were found to contain bacteriophage. It appeared feasible to determine the duration of bacteriophage in a sensitive culture of *Streptococcus lactis* and to study some of the characteristics of the secondary-growth organisms resulting from the action of bacteriophage.

Nichols and Ineson (4) attributed starter recovery in commercial cultures to a mixture of several strains in the starter, some strains being unaffected by bacteriophage. Hunter (2) stated that the mechanism by which resistant forms originate after lysis of a culture by phage has not been explained satisfactorily. Yakovlev (5) noted that secondary-growth cultures were temporarily resistant to bacteriophage when grown on solid media; on liquid media their resistance was permanent but they remained carriers of bacteriophage. Hunter (2) found that under certain circumstances a phage-coccus mixture could be subcultured daily in milk and lysis did not occur. Such cocci, in the presence of symbiotic phage, showed resistance to lysis by other phage strains. Nelson *et al.* (3) state that the inhibitory principal (bacteriophage) may have a destabilizing effect on the cell when the changes involved in the loss of sensitivity characteristics are effected. The destabilized cell then may undergo either a gain or loss of sensitivity to various filtrates, the direction of the change being conditioned by factors at present unknown.

## EXPERIMENTAL METHODS

*Propagation of cultures.* Cultures of *S. lactis* were transferred in skim milk which had been autoclaved for 15 min. at 15 lb. pressure. An inoculation of approximately 1.0 per cent was employed.

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*Determination of bacteriophage titer.* Bacteriophage titers were determined on bacteria-free filtrates by the serial dilution method (1). The bacteriophage titer was expressed as the milliliters of filtrate required to cause a retardation in the production of acid, reduction of litmus or coagulation of milk by a sensitive culture of *S. lactis*.

*Preparation of bacteria-free filtrates.* Bacteria-free filtrates of cultures were prepared by filtering the coagulated milk cultures, under aseptic conditions, through coarse filter paper and then passing the filtrate thus obtained through a Selas micro-porous filter of #03 porosity.

TABLE 1

*Coagulation times and bacteriophage titers of a culture of S. lactis, without and with added bacteriophage*  
(Incubated at 21° C.)

Transfer no.	Time required to obtain a firm coagulum (hr.)			Bacteriophage titer of culture receiving one inoculation with bacteriophage
	Control culture. Free of bacteriophage	Culture receiving one inoculation with bacteriophage	Culture receiving inoculation with bacteriophage at each transfer	
1	24	48	48	10 <sup>-9</sup>
2	18	23	23	10 <sup>-8</sup>
3	18	22	22	10 <sup>-7</sup>
4	18	18	19	10 <sup>-4</sup>
5	19	19	22	10 <sup>-6</sup>
15	22	24	22	10 <sup>-5</sup>
25	20	20	20	10 <sup>-8</sup>
35	21	27	27	10 <sup>-5</sup>
45	17	18	18	10 <sup>-4</sup>
55	19	19	20	10 <sup>-8</sup>
65	17	17	17	10 <sup>-8</sup>
75	17	17	17	10 <sup>-1</sup>
85	17	20	17	10 <sup>-1</sup>
95	15	15	15	10 <sup>-1</sup>
105	15	15	15	10 <sup>-1</sup>
115	16	16	16	10 <sup>-1</sup>
125	16	16	16	10 <sup>-2</sup>
132	16	16	16	< 1
133	15	15	15	< 1
134	15	15	15	< 1
135	15	15	15	< 1

*Selection of test cultures.* The pure cultures selected for study were rapid acid-producing strains isolated from commercial cultures. Only cultures showing rapid secondary growth were included in the study.

## RESULTS

The coagulation times of a culture maintained in a bacteriophage-free condition, the coagulation times and bacteriophage titers of the same culture inoculated once with bacteriophage active against the organism and the coagulation times of the same culture given an inoculation with bacteriophage at each transfer are given in table 1.

The first transfer of the control culture (bacteriophage-free) coagulated milk 24 hr. sooner than the same culture inoculated with bacteriophage when an in-

cubation temperature of 21° C. was employed. The second transfer of the control culture coagulated milk 5 hr. sooner and the third transfer 4 hr. sooner than the cultures containing bacteriophage. Only slight variations in the coagulation times of all cultures were noted on the fourth and succeeding transfers. The culture inoculated once with bacteriophage had approximately the same coagulation time as the culture inoculated with bacteriophage at each transfer. The bacteria-free filtrate used in this experiment had a bacteriophage titer of  $10^{-6}$  and the cultures were given a 1 per cent inoculation of the filtrate.

The bacteriophage titer of the culture receiving one inoculation with bacteriophage was  $10^{-9}$  on the first transfer and showed a general decrease with succeeding propagations. However, the bacteriophage titer remained rather

TABLE 2  
*Coagulation times and bacteriophage titers of a culture of S. lactis, without and with added bacteriophage*  
(Incubated at 26° C. for 9 transfers and at 21° C. for the remaining transfers)

Transfer no.	Time required to obtain a firm coagulum (hr.)			Bacteriophage titer of culture receiving one inoculation with bacteriophage
	Control culture. Free of bacteriophage	Culture receiving one inoculation with bacteriophage	Culture receiving inoculation with bacteriophage at each transfer	
1	12	30	30	$10^{-9}$
2	14	20	20	$10^{-9}$
3	13	20	24	$10^{-6}$
4	14	18	18	$10^{-8}$
5	14	18	18	$10^{-6}$
6	14	17	20	$10^{-6}$
7	15	17	17	$10^{-6}$
8	14	12	12	$10^{-6}$
9	12	12	15	$10^{-6}$
10	12	14	14	$10^{-6}$
15	18	21	22	$10^{-6}$
20	21	22	21	$10^{-4}$
25	18	18	22	$10^{-5}$
30	18	18	18	$10^{-5}$
33	18	18	18	$10^{-3}$
34	20	20	20	$10^{-3}$
35	23	23	23	$< 1$
36	22	22	22	$< 1$
37	22	24	22	$< 1$

constant from the 75th to the 115th transfer; during this period the titer was low ( $10^{-1}$ ). No bacteriophage could be detected in 1 ml. of a bacteria-free filtrate after 132 transfers.

The experimental data presented in table 2 were obtained with the same culture used in obtaining the data for table 1. Also, the experiment was carried out in the same manner except that the cultures were incubated at 26° C. for nine transfers and thereafter at 21° C. Table 2 shows that the first transfer of the control culture (bacteriophage-free) coagulated milk 18 hr. sooner than the same culture inoculated with bacteriophage. The difference in coagulation time between the control culture and the cultures containing bacteriophage became smaller with succeeding propagations. The culture receiving one inocula-

tion with bacteriophage, as well as the culture receiving an inoculation with bacteriophage at each transfer, coagulated milk in less time than the control culture on the eighth transfer. There were only small variations in the coagulation times of all cultures after the eighth transfer.

The bacteriophage titer of the culture receiving one inoculation with bacteriophage was  $10^{-9}$  on the first and second transfers and then decreased. From the sixth to the 15th transfer the bacteriophage titer remained constant and then decreased. No bacteriophage could be detected in 1 ml. of bacteria-free filtrate after the 35th transfer. The bacteria-free filtrate used in this trial had a bacteriophage titer of  $10^{-6}$  and the cultures were given a 1 per cent inoculation.

The data presented in table 3 give the coagulation times and bacteriophage titers of a culture of *S. lactis*, without and with added bacteriophage when incubated at 37° C. The culture employed in this trial was the one used to obtain the data presented in previous tables. Table 3 shows that the first transfer of

TABLE 3

*Coagulation times and bacteriophage titers of a culture of S. lactis, without and with added bacteriophage*  
(Incubated at 37° C.)

Transfer no.	Time required to obtain a firm coagulum (hr.)			Bacteriophage titer of culture receiving one inoculation with bacteriophage
	Control culture. Free of bacteriophage	Culture receiving one inoculation with bacteriophage	Culture receiving inoculation with bacteriophage at each transfer	
1	5	76	76	$10^{-7}$
2	6	23	23	$10^{-6}$
3	7	24	26	$10^{-6}$
4	15	22	24	$10^{-6}$
5	15	15	15	$10^{-6}$
6	12	15	15	$10^{-6}$
7	24	24	32	$10^{-1}$
8	24	24	24	$10^{-4}$
9	12	19	20	$10^{-3}$
10	15	19	19	$10^{-1}$
11	15	19	19	< 1
12	16	16	16	< 1
13	16	15	15	< 1
14	10	12	12	< 1

the control culture (bacteriophage-free) coagulated milk 71 hr. sooner than the culture inoculated with bacteriophage. Marked differences in the coagulation times occurred on the second and third transfers also, but on the fifth transfer the coagulation times were the same for all cultures. Some variations in the coagulation times of the cultures were evident after five transfers but they were not great. The coagulation time of the control culture increased slightly for three transfers and an appreciable increase took place on the fourth propagation. The control culture had the greatest coagulation time on the seventh and eighth transfers and then the coagulation time decreased with some fluctuation.

The bacteriophage titer of the culture receiving one inoculation with bacteriophage was  $10^{-7}$  on the first transfer and  $10^{-6}$  on the second to fifth transfers.

The bacteriophage titer showed a general decrease with some variation until the 11th transfer. No bacteriophage was detected in 1 ml. of filtrate after the 11th transfer. The bacteria-free filtrate used to inoculate the cultures had a bacteriophage titer of  $10^{-5}$  and the cultures were given a 1 per cent inoculation.

The bacteriophage titers of the cultures receiving an inoculation with bacteriophage at each transfer were determined but were not reported in the tables. In general, the bacteriophage titers of these cultures were relatively high for the first few transfers and then approached a value approximately equal to the titer of the bacteria-free filtrate added, taking into consideration the amount of dilution by the skim milk employed as the growth medium. Also, the coagulation times and bacteriophage titers were determined on each transfer of a culture, even though all are not reported. The close correlation of results from day to day made it appear unnecessary to give data for each transfer.

The data presented in table 1 show that the bacteriophage titer of the culture receiving one inoculation with bacteriophage was  $10^{-1}$  from the 75th to the 115th transfer and that the culture was not free of bacteriophage until the 132nd transfer. On the 113th transfer, two lots of the same milk were inoculated with the culture which received an initial inoculation with bacteriophage. One lot was incubated at 21° C. (data in table 1) and the other at 37° C. The culture incubated at 37° C. did not contain bacteriophage in 1 ml. of bacteria-free filtrate after one propagation, while the culture incubated at 21° C. showed bacteriophage present for an additional eighteen propagations. The results indicated that a culture carrying a small amount of bacteriophage for a rather long period of time at 21° C. quickly lost the bacteriophage when the incubation temperature was increased to 37° C.

The secondary-growth organisms resulting from the action of bacteriophage on a sensitive culture of *S. lactis* did not appear to undergo a change in morphology or colony characteristics. Sugar fermentations by the secondary-growth organisms were the same as for the parent culture. The secondary-growth organisms (free of bacteriophage) were not sensitive to the original bacteriophage which had caused lysis previously. The culture used to obtain the data presented in table 2 was used in a commercial cheese plant after it became free of bacteriophage with repeated transfer. The cheese plant selected was one which previously had difficulty with slow acid production due to bacteriophage. Although this culture was not sensitive to the original bacteriophage (phage #1) which had caused lysis, the culture developed sensitivity to another bacteriophage (phage #2) after it had been used in the cheese plant on 3 successive days. When phage #2 was added to another transfer of this culture that was carrying phage #1, no inhibition of the culture was evident, but when this culture became free of bacteriophage after repeated transfer, it became sensitive to the newly acquired strain of bacteriophage (phage #2).

#### DISCUSSION

The data reported in these experiments may help to clarify the instances of slow acid production, of rather short duration, frequently encountered in plants

where mother cultures are propagated daily for use in the manufacture of various dairy products. The results indicate that some cultures show a rather rapid secondary growth after attack by bacteriophage and behave quite normally after a few transfers. When a mother culture fails in a commercial plant which is carrying several cultures, it frequently is assumed that the culture was not inoculated by the person making the transfer. If only one culture is carried, it generally is thought that the culture became contaminated, that the incubator used may have failed to heat properly during the night and the temperature was too cold, etc. Many failures of mother cultures can be explained on the basis of bacteriophage.

In all of the experiments conducted, sterile skim milk was employed as the growth medium. The sterile skim milk showed various degrees of "browning" after sterilization and it was noted generally that the cultures required a slightly longer period of incubation to form a firm clot in the lots of skim milk showing the most "browning." Therefore, slight differences in coagulation times are not significant. All cultures transferred on a particular day were from the same lot of milk and the comparisons between cultures receiving various treatments can be made with the control culture.

The appearance of secondary-growth organisms was more rapid at 26° C. than at 21 or 37° C. The results indicate that the rate of growth of the secondary-growth organisms is not related to the optimum growth temperature. The control culture, on the first propagation, coagulated milk in 24 hr. at 21° C., in 12 hr. at 26° C. and 5 hr. at 37° C.

The data presented in table 3 show that the control culture became progressively slower in acid production for the first eight transfers at 37° C. Later, it became somewhat more active. The culture used in these trials had been incubated at 21° C. previous to the time of these experiments. It has been noted that a culture carried at 21° C. does become somewhat slow when incubated at a higher temperature. Also, such a culture may not show its normal rate of acid production when returned to the lower temperature.

Studies on the secondary-growth organisms indicate that a culture which is carrying bacteriophage and producing acid quite normally may not be attacked by another bacteriophage strain until it becomes free of bacteriophage. The presence of bacteriophage in a culture may prevent a further attack by another strain of bacteriophage.

#### SUMMARY AND CONCLUSIONS

A study was made of the coagulation times and bacteriophage titers of a culture of *S. lactis*, without and with added bacteriophage, using incubation temperatures of 21, 26 and 21, and 37° C.

A culture of *S. lactis* inoculated once with bacteriophage active against the culture required 132 transfers before the culture became free of bacteriophage when incubation was carried out at 21° C. When the culture was inoculated once with bacteriophage and incubated at 26° C. for nine transfers and then at 21° C., it did not contain bacteriophage after 34 transfers. The same culture



inoculated once with bacteriophage and propagated at 37° C. did not contain bacteriophage after 10 transfers.

Cultures of *S. lactis* inoculated with bacteriophage active against the cultures produced acid rather rapidly after a few transfers, even though bacteriophage was present in the cultures.

Secondary-growth organisms resulting from the action of bacteriophage on a culture of *S. lactis* appeared to be similar to the parent culture in morphology, colony characteristics and in their ability to ferment sugars.

Secondary-growth organisms were resistant to the bacteriophage type causing incomplete lysis of the parent culture but were sensitive to at least one other bacteriophage type.

Secondary-growth organisms propagated in the presence of bacteriophage active against the parent culture were not sensitive to another bacteriophage type until the original bacteriophage had disappeared from the culture after repeated transfer.

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## INFLUENCE OF CRUDE FIBER IN THE RATION ON EFFICIENCY OF FEED UTILIZATION BY DAIRY COWS<sup>1</sup>

SAM NORDFELDT, ISAAC IWANAGA, K. MORITA, L. A. HENKE AND  
ANNIE K. S. TOM

*University of Hawaii Agricultural Experiment Station, Honolulu, T. H.*

### INTRODUCTION

Roughages commonly fed to dairy cows in Hawaii tend to be high in fiber. One of these roughages is "strip cane," and another is "cane tops," both of which are obtained as waste or by-products from sugar plantations. Near Honolulu other roughages are grown as soiling crops (Napier grass, *Pennisetum purpureum*) or obtained in wild state from gulches and other waste areas (koa haole, *Leucaena glauca*). These and other roughages are unusually high in crude fiber. For example, strip cane contains 44 per cent crude fiber, sugar cane tops, 37 per cent, Napier grass, 41 per cent and koa haole, 37 per cent crude fiber when calculated on the dry matter basis. Therefore, in this area, it is very important that an investigation be made as to the correct balance of crude fiber to total digestible nutrients in the rations fed to cattle.

### REVIEW OF LITERATURE

Many investigators have worked on various phases of nutrition closely related to the problem mentioned above. Henke (6) reported that the 4 per cent F.C. milk production was 25.4 lb. per cow daily when the average concentrate consumption was 19.8 lb. as compared to 24.1 lb. when concentrate consumption averaged 15.4 lb. Napier grass was fed as the roughage. In another experiment the same author (5) studied pineapple tops as a substitute for Napier grass when the concentrate rations were kept equal. The 4 per cent F.C. milk yield averaged 5.5 per cent higher when pineapple tops were fed. The amount of crude fiber calculated on the percentage of dry matter in the feed was 12.4 per cent as compared to 21.1 per cent when Napier grass was fed. However, the content of digestible protein was slightly higher in the pineapple tops group. At the Massachusetts Station high concentrate feeding also was compared with rather low allowance of concentrates and a maximum amount of roughage (8). On a high concentrate ration the milk yield was 31.7 lb. daily, as compared with 27.7 lb. for the ration containing a smaller amount of concentrates. This experiment, as well as those carried out at the Hawaii Agricultural Experiment Station, was concerned primarily with the economic phase of this type of feeding.

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As to the physiological optimum of crude fiber in the rations, relatively little work has been done. Working with fattening lambs, Cox (4) found that when using the proportions 35:65, 45:55 and 55:45 of concentrates to roughage, the best gain in weights always was obtained for the 45:55 ratio. The concentrates in this, as in most of the other experiments, consisted of corn; in some of the experiments cottonseed meal also was used. The roughage consisted of alfalfa meal or alfalfa hay and Atlas silage.

Other workers have obtained similar results for growing pigs. In one series of experiments, increasing amounts of oats were used with corn and in another series, increasing amounts of ground wheat straw were used with corn (10). The intake of metabolizable energy and necessary nutrients in the different groups were kept equal; the only difference between groups was the increasing amount of crude fiber or roughage. In the oats-corn series the best gain in the pigs was obtained in the group receiving 75 per cent oats and 25 per cent corn. In the wheat straw meal-corn series, the best result was obtained in the group receiving 7 per cent wheat straw meal and 93 per cent corn.

A number of other experiments have been carried out from time to time dealing with different phases of nutrition closely related to this problem, and Cox has given a review of them (4). He finds, however, that work pointed specifically to determination of the significance of this factor (by Cox named the "physical balance") has been sketchy and has lacked continuity. Therefore, it appears important that investigations of this type be carried out with milking cows and possibly also with beef cattle.

#### PLAN OF EXPERIMENT

Four lots of three cows each from the station dairy herd were used for the experiment. The cows were of the Holstein-Friesian breed with an age averaging close to 7 yr. The number of lactation days averaged 127 and the daily milk yield per cow, 32.6 lb., with a fat percentage of 3.48. Four per cent F.C. milk averaged 30 lb. daily per cow. The weight of the cows averaged 1,182 lb. at the start of the experiment. From the averages given above, it is evident that variations occurred among individual cows. Therefore, it was necessary to design the experiment in such a way that errors in final results due to variation among cows could be excluded as far as possible.

The four lots of cows were used during a 16-wk. change-over design for four rations containing decreasing amounts of crude fiber. The lots were divided at random after the age, weight, milk production and days since calving were considered. The four rations were designated by the letters A, B, C and D according to the following Latin square scheme, as suggested by Cochran *et al.* (3):

Period	Cows				Cows				Cows			
	1	2	3	4	5	6	7	8	9	10	11	12
I	A	B	C	D	A	B	C	D	A	B	C	D
II	B	A	D	C	D	C	B	A	C	D	A	B
III	C	D	A	B	B	A	D	C	D	C	B	A
IV	D	C	B	A	C	D	A	B	B	A	D	C

This design means that for each period each one of the four rations is tested on three cows. By the end of the experiment the four rations are tested on all 12 cows.

The composition of the concentrate rations used is given in table 1.

TABLE 1  
*Concentrate Mixtures*

Mixture no.	Cane molasses	Pineapple bran	Soybean oil meal	Meat meal	Fish meal	Salt	Bone meal	Sum
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
15a	250	430	200	50	50	10	10	1000
15b	250	500	130	50	50	10	10	1000
15c	250	540	90	50	50	10	10	1000
15d	250	575	55	50	50	10	10	1000

The chemical composition of these mixtures and also of other feeds used in the experiment is given in table 2.

TABLE 2  
*Percentage chemical composition of feed and concentrate mixtures*

	Moisture	Protein	Ether extract	Crude fiber	Ash	N-free matter	Calculated	
							D.P.	T.D.N.
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Napier grass	75.57	1.28	0.47	9.02	2.98	10.68	0.77	14.61
Molasses	20.30	2.27			11.16	66.26	0.73	59.67
Conc. mixture 15a	12.70	16.08	2.60	8.83	8.69	51.10	11.95	64.93
“ “ 15b	12.52	12.72	2.53	9.75	8.38	54.10	9.02	65.97
“ “ 15c	12.71	11.16	2.57	10.34	9.03	54.19	7.29	65.46
“ “ 15d	13.37	10.46	2.67	10.46	8.61	54.43	6.84	64.34
Soybean oil meal	10.25	43.02	4.16	5.65	5.39	31.53	36.56	79.06

The roughage used consisted of mature Napier grass produced on the station's dairy farm. Before feeding, the grass was always chopped into pieces of 1 to 2 in. in length. The amounts of roughage used in rations A, B, C and D were 60, 40, 20 and 0 lb., respectively.

The calculated composition of the complete rations is presented in table 3. In this example, the average milk production of the cows was 28 lb. daily of 4 per cent F.C. milk.

In planning the rations, the main concern was to obtain, as far as possible, equal amounts of digestible protein and total digestible nutrients in each of the ration for the same level of milk production. Sufficient amounts of carotene and minerals were provided in different rations. The only variable factor was the crude fiber content. The four different concentrate mixtures were composed, one for each ration, to simplify the feeding work. At lower or higher levels of production, some of the mixtures needed supplements of a slight amount of soybean oil meal to raise the protein content to a suitable level. Table 3 shows that nutrients provided in different rations parallel each other as closely as possible. The crude fiber in the rations is slightly lower than the 24, 22, 18 and 14 per cent originally planned for rations A, B, C and D, respectively.

TABLE 3  
Composition of rations

Feed	Amount	Dry matter	D.P.	T.D.N.	Nutri- tive ratio	Ether extract	Crude fiber	Crude fiber of D.M.
	(lb.)	(lb.)	(lb.)	(lb.)		(lb.)	(lb.)	(%)
<i>Ration A</i>								
Napier grass	60.0	14.658	0.462	8.765		0.279	5.412	
Conc. #15a	13.7	11.960	1.637	8.895		0.356	1.210	
Molasses	3.0	2.390	0.022	1.790				
Soybean oil meal	0.3	0.269	0.110	0.237		0.012	0.017	
Sum		29.28	2.231	19.69	1: 9	0.647	6.64	22.7
Requirement*			2.134	18.36				
<i>Ration B</i>								
Napier grass	40.0	9.772	0.308	5.844		0.188	3.608	
Conc. #15b	18.8	16.446	1.696	12.402		0.475	1.832	
Molasses	2.0	1.594	0.015	1.193				
Sum		27.81	2.019	19.44	1: 10	0.663	5.44	19.6
Requirement			2.134	18.36				
<i>Ration C</i>								
Napier grass	20.0	4.886	0.154	2.922		0.094	1.804	
Conc. #15c	23.6	20.600	1.720	15.448		0.606	2.441	
Molasses	1.0	0.797	0.007	0.597				
Soybean oil meal	0.3	0.269	0.110	0.237		0.012	0.017	
Sum		26.55	1.991	19.20	1: 10	0.712	4.27	16.1
Requirement			2.134	18.36				
<i>Ration D</i>								
Conc. #15d	28.6	24.776	1.955	18.401		0.763	2.990	
Soybean oil meal	0.3	0.269	0.110	0.237		0.012	0.017	
Sum		25.05	2.065	18.64	1: 9	0.775	3.01	12.0
Requirement			2.134	18.36				

\* Requirement calculated for cows weighing 1,200 lb.

All concentrate was fed individually. For practical purposes, the roughage was weighed out daily to each lot of three cows. Normally all feed was eaten. In cases where leftovers occurred, these were weighed back and the net consumption of feed recorded.

All ingredients in the concentrate mixtures were sampled and analyzed for dry matter, ash, protein, fat, crude fiber and N-free extract. For control, samples also were taken of the concentrate mixtures and analyzed as above for each separate mixture. A sample of the Napier grass used was taken each day and analyzed for dry matter content. The daily samples were composited and chemical analysis as above carried out for each period. Results of the analysis are given in table 2.

All milk produced by the cows was weighed daily. The fat test of the milk was taken during Monday afternoon, Tuesday morning and afternoon and Wednesday morning each week. A Babcock tester was used.

After the milk and fat test for each week was finished, the amount of concentrate to be fed during the week to follow was calculated. The feed intake

was equalized for different groups according to milk production. Morrison's feeding standards for good cows under usual conditions were followed.

Weight of animals was taken before and at the end of each period during three successive daily weighings. As it appeared worthwhile to follow the general well being and physical condition of the animals fed the different rations as closely as possible, pulse, temperature and respiration rate measurements were taken weekly. These readings always were started at 3:30 p.m. before milking of the cows. For comparison of night and day records, data also were assembled in the mornings, readings starting at 4:00 a.m. The morning readings were continued for 5 wk. Temperature was obtained with a so-called veterinary thermometer inserted into the rectum approximately 3 in. and left for 3 min. Respiration rate was determined by number of flank movements per minute. In determining pulse rate, the tips of the fingers were placed on the under side of the tail where the movements per minute of the coccygeal artery were counted.

#### RESULTS

Generally, the course of the experiment proceeded according to plan. During some of the experimental days, particularly during the last week of June, rather high climatic temperatures apparently influenced the well being and milk production of the cows. This incident did not interfere with the final results of the experiment.

For one cow (no. 175), abnormally low milk production was obtained during the last period of the experiment, particularly during the last week. From 17 lb. of milk daily in the beginning of this period, the yield decreased to 8 lb. and low production then continued until the dry state was reached. Therefore, normal data for this cow during the period mentioned are missing. By use of Snedecor's (13) formula, the missing value was calculated. The original incorrect value is given within parenthesis in table 4 and the calculated value is marked with an asterisk.

During the whole of the experiment, consumption of the different rations used was good. Only for the ration A (including 60 lb. Napier grass daily) a few weigh-backs occurred; of 15,020 lb. of this roughage fed, less than 0.5 per cent of the total was not eaten. In the groups fed 40 and 20 lb. roughage daily, all roughage was consumed. Slight leftovers occasionally occurred during the first week of each period, after the switchover from one ration to another had taken place. All concentrate was eaten, except for ration D, where 7 lb. were left by one cow and 4.5 lb. by another one during the first part of period I.

In order to minimize carry-over effects from one period to a following, the data for the first week in each period are omitted in the following calculations. By this omission the source of error introduced by the leftovers mentioned above also is excluded.

The results in milk yield for the different rations are collected in table 4.

After analysis of variance of the data for milk yield presented in table 4, the analytical results were assembled in table 5. By examination of the mean squares, it is found that results obtained for points 1 to 4 listed in the table are

TABLE 4  
4% F. C. milk yield, in pounds, obtained for different rations  
(One seventh of the milk production for each period is given in the table)<sup>a</sup>

Period	Group 1				Group 2				Group 3						
	Cows				Cows				Cows						
	226	289	292	172	Total	277	175	219	176	Total	192	287	259	224	Total
I	A 96	B 97	C 85	D 91	369	A 57	B 88	C 85	D 80	310	A 58	B 67	C 74	D 58	257
II	B 86	A 83	D 83	C 86	338	D 62	C 82	B 65	A 65	274	C 62	D 65	A 62	B 50	239
III	C 82	D 85	A 64	B 65	296	B 51	A 58	D 67	C 59	235	D 60	C 56	B 63	A 45	324
IV	D 75	C 80	B 62	A 55	272	C 43	D 65 <sup>b</sup>	A 57	B 39	204	B 50	A 44	D 64	C 50	208
							(36)								
Totals	339	345	294	297	1,275	213	293	274	243	1,023	230	232	263	203	928
Treatment totals:															
	Sum				Daily av.	Sum				Daily av.	Sum				Daily av.
	A = 298				24.83	A = 237				19.75	A = 209				17.42
	B = 310				25.83	B = 243				20.25	B = 230				19.17
	C = 333				27.75	C = 269				22.42	C = 242				20.17
	D = 334				27.83	D = 274				22.83	D = 247				20.58

<sup>a</sup> The reason that only one seventh of the milk production for each period is given, and not the total sum, is due to the fact that giving the same final result this method decreases the labor needed in successive calculations.

<sup>b</sup> This figure was calculated according to the formula for missing data in a Latin square, as reported by Snedecor (13).

highly significant. The mean square for between rations is 228.25 and the mean square for errors is 21.10. The  $F$ -value, therefore, is  $228.25/21.10 = 10.82$ . According to Snedecor's tables (13) for the distribution of  $F$ , an  $F$ -value of only 5.18 is needed in this case for significance at the 1 per cent point.

It should be mentioned that a test for significance also was carried out with use of the original milk yield value for cow 175 in period 4 without making use of the above-mentioned corrected value. In this case, an  $F$ -value of 4.52 was obtained, which still is sufficient for significance close to the 1 per cent point.

TABLE 5  
*Analysis of variance of 4% fat corrected milk units (lb.)*

	Degrees of freedom	Sum of squares	Mean squares
1. Between groups	2	4019.54	2009.77
2. Between cows within groups	9	1925.38	213.93
3. Between periods within groups	9	3324.38	369.38
4. Between rations	3	684.75	228.25
5. Ration $\times$ group interactions	6	21.13	3.52
6. Error	17 <sup>a</sup>	358.74	21.10
7. Total	46 <sup>a</sup>	10333.92	

<sup>a</sup> One degree of freedom subtracted for cow 175.

It is clear, therefore, that the amount of crude fiber in the rations tested had a definite influence upon the milk yield. This is graphically illustrated in figure 1, where the average daily milk yields in pounds obtained for the different rations tested are plotted against the crude fiber content of the feed. The curve in the graph is fitted by free hand. It is seen that the yield of milk definitely decreased as soon as the crude fiber content in the feed increased over 16 per cent, calculated on the dry matter basis.

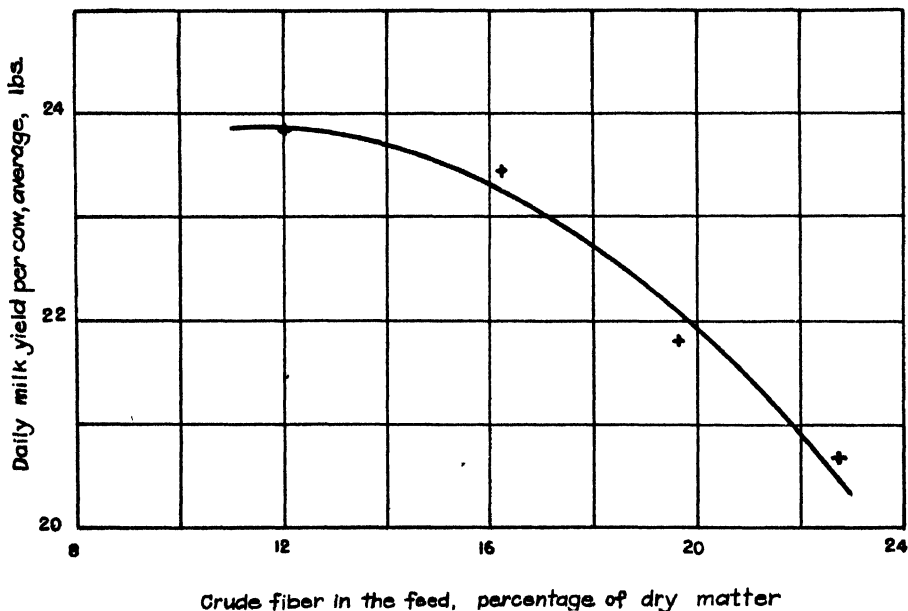
*Effect of different rations on weights of cows during experiment.* The average body weights for the cows on rations A, B, C and D were, at the start of the first period, 1,177, 1,154, 1,141 and 1,255 lb., respectively; at the start of the second period, 1,182, 1,157, 1,126 and 1,148 lb.; at the start of the third period, 1,196, 1,145, 1,266, 1,006 lb.; at the start of the fourth period, 1,121, 1,237, 1,211, 1,085 lb.; and at the end of the fourth period, 1,234, 1,169, 1,061 and 1,214 lb. The average weight of all cows at the beginning of the experiment was 1,182 lb.

At the end of the experiment the average weight was 1,170 lb. Each figure was based upon weighings taken during 3 successive days. Therefore, it appears that a loss in weight of 12 lb. per cow occurred during the 16 wk. of experiment. This loss is very truly a result of the more concentrated feed for part of the cows and could hardly be ascribed to a real loss in body weight. An example will illustrate this. Cow no. 172 had an initial weight of 1,401 lb. when she started on ration D. After 4 wk. on this concentrated ration her weight was 1,266 lb. From this ration she was turned over in period II to the slightly less concentrated ration C. At the end of this period her weight had increased to 1,291 lb. From this ration she was fed the still less concentrated ration B in period III, and an increase in weight to 1,347 lb. resulted. In the last period she was fed



ration A and this increased her weight to 1,411 lb. at the end of the experiment.

Since these differences in body weight occurring over a short time could not be due to real gain in body tissues, they must be ascribed to different weights of the digestive channel content. According to Sisson and Grossman (12), the stomachs of large cattle have a capacity of 40 to 60 gal. and those of medium size, 30 to 40 gal. This would correspond to about 40 gal. or 150 l. for the cows used in this experiment and illustrates the large feed capacity of the cows.



An examination of the rations used in the experiment listed in table 1 reveals that for a cow milking 28 lb. daily, ration A for 1 day weighed 77 lb.; ration B, 61 lb.; ration C, 45 lb. and ration D, 29 lb. Although these rations contain the same amount of T.D.N. and D.P. and although the difference in dry matter content is not more than 4.23 lb. between rations A and D, it is obvious that the coarser nature of rations A and B must affect the weight of the animals, as borne out in the example for cow no. 172. This study illustrates the danger in emphasizing changes in weight occurring in experiments of this type.

*Effect of different rations upon pulse rate.* In order to study whether the type of feeding had any influence upon the pulse rate of the cows, the afternoon readings were collected in table 6. After analysis of variance of the pulse rates presented in table 6, the analytical results were assembled in table 7. The mean square for between rations is 136.81 and for the error, 30.07. The  $F$ -value for between rations, therefore, is  $136.81/30.07 = 4.55$ . In Snedecor's tables for the distribution of  $F$  with  $n^1 = 3$  and  $n^2 = 18$ , the value of  $F$  for the 5 per cent point is 3.16 and for the 1 per cent point, 5.09. As is seen, the significance comes closer

TABLE 6

*Pulse rate of cows at afternoon readings  
(Average for last 3 wk. of each period)*

Pulse rate of cows <i>av.</i> (Average for last 3 wk. of each period)																
Period	Group 1				Group 2				Group 3							
	Cows		Total	Cows		Total	Cows		Total	Cows		Total				
	286	289		292	172		272	175		219	176		192	237	259	274
						Pulse-rate/min. <sup>a</sup>										
I	A 64	B 76	C 69	D 61	270	A 68	B 72	C 64	D 67	271	A 69	B 76	C 68	D 55	268	
II	B 65	A 69	D 57	C 74	265	D 78	C 68	B 62	A 64	272	C 70	D 63	A 59	B 75	267	
III	C 72	D 60	A 76	B 73	281	B 72	A 80	D 59	C 68	279	D 65	C 67	B 69	A 72	273	
IV	D 59	C 61	B 53	A 60	233	C 77	D 71	A 53	B 72	273	B 71	A 64	D 52	C 71	258	
Totals	260	266	255	268	1,049	295	291	238	271	1,095	275	270	248	273	1,066	
Treatment totals:																
	Sum		Daily av.			Sum		Daily av.			Sum		Daily av.			
	A = 269		67.3			A = 265		66.3			A = 264		66.0			
	B = 267		66.8			B = 278		69.5			B = 291		72.8			
	C = 276		69.0			C = 277		69.3			C = 276		69.0			
	D = 237		59.3			D = 275		68.8			D = 235		58.8			

<sup>a</sup> The figures mean average pulse rate for the last 3 wk. of each period.

TABLE 7

*Analysis of variance of pulse rate per minute for experimental cows*

	Degrees of freedom	Sum of squares	Mean squares
1. Between groups	2	67.63	33.82
2. Between cows within groups	9	652.12	72.46
3. Between periods within groups	9	357.62	39.74
4. Between rations	3	410.42	136.81
5. Ration $\times$ group interactions	6	262.20	43.70
6. Error	18	541.26	30.07
7. Total	47	2291.25	

to the 1 than to the 5 per cent point. This means that the rations used in this experiment did influence the pulse rate of the cows.

We are not aware from earlier reports that increase in roughage fed to milk cows would affect the pulse rate, although Thomas (14) recently has reported that feeding 120 to 130 per cent of required T.D.N. to producing cows increases the heart rate. Exercise or carrying a burden increases the heart rate and it appears that the increase in roughage fed to cows is a parallel to that phenomenon.

*Respiration rate.* The data obtained for respiration rate were treated in the same way as were the data for pulse rate. No significant influences of rations upon respiration rate occurred. The *P*-value obtained for this influence fell between 0.2 and 0.05.

The afternoon respiration rate varied from 24 to 68 per minute, with an average of  $37.8 \pm 3.32$ .

*Body temperature.* Similarly, body temperature reflected no significant influence of types of rations. Variations in afternoon body temperature were from 100.0 to 102.8° F. Average body temperature was  $101.3 \pm 0.182$ ° F.

*Morning readings versus afternoon readings for pulse rate, respiration rate and body temperature.* In table 8 the averages of pulse rate, respiration rate and

TABLE 8

*Averages of pulse rates, respiration rates and body temperatures*

	No. of observations	Mean	Standard error	Lowest value observed	Highest value observed
<i>Morning readings</i>					
Pulse rate/min.	60	72.2	$\pm 1.446$	52	104
Respiration rate/min.	60	32.4	$\pm 1.155$	22	66
Body temperature (° F.)	60	101.5	$\pm 0.065$	100	102.5
<i>Afternoon readings</i>					
Pulse rate/min.	180	66.3	$\pm 3.470$	48	88
Respiration rate/min.	192	37.8	$\pm 3.320$	24	68
Body temperature (° F.)	192	101.3	$\pm 0.182$	100	102.8

body temperature measured at 4 to 5:15 in the mornings as compared to measurements at 3:30 to 4 in the afternoon are given. The readings in the mornings were taken to see whether or not the surrounding air temperature would influ-

ence the characters measured. The lowest air temperature at night was about 15° F. below the highest temperature during the day. Minimum temperature for the nights when pulse rates were taken averaged 70.8° F., whereas maximum temperatures in afternoons of the same days averaged 85° F.

In table 8 an indication is given that the pulse rate decreases with increase in temperature. This is in agreement with results of Kleiber and others, as reported by Brody (2). Due to the lack of sweat glands in species such as cows and pigs, the skin is not cooled by sweating and therefore less blood is sent to the surface when environmental temperature rises. However, the difference in rate for morning and afternoon readings as reported here ( $72.2 - 66.3 = 5.9^{\circ}$  F.) is not sufficient for statistical significance. Neither is there any significant difference between morning and afternoon readings of respiration rate and body temperature.

#### DISCUSSION

The influence of percentage of crude fiber in the feed upon milk production is clearly demonstrated. A number of experiments have been carried out where the amount of roughage and its influence upon the milk production has been studied. In most cases of this work, the emphasis is put upon the character of roughage as such and less on the fiber content of the feed.

Morrison (9) discusses milk production on roughage alone and also the effect of successive additions of concentrate, and refers to a number of feeding experiments. As the level of concentrate feeding was increased in these experiments, the amount of additional milk secured per pound of concentrate decreased steadily. The decrease in milk per pound of concentrate fed was from 1.3 lb. milk at a high level of roughage to 0.3 lb. at a low level. The highest level of roughage was taken as 11,338 lb. of hay or "hay equivalent" fed per cow per year. The low level was taken as 7,385 lb. of hay or "hay equivalent" per year. However, the milk yields in different groups were not strictly comparable because at the higher levels of concentrates the digestible nutrient intake was about 16 per cent above the standard used, while in the medium levels of concentrate feeding it was only "a trifle" more than advised in the standards used. Probably different results would have been obtained if all the groups had been fed at the same level of total digestible nutrients and protein.

Among workers who have suggested that the feeding of milk cows is regulated according to crude fiber content of the feed, is Axelsson (1). He believes that the amount of crude fiber in the feed given shows an optimum value. Logically such an optimum occurs. As mentioned before, Cox has observed such an optimum in experiments with fattening lambs. The best gains in weight of the lambs were obtained for the 45:65 ratio of concentrate to roughage. In our experiment the best milk production was obtained when the fiber content was decreased to 16 per cent or below (calculated on dry matter basis). All rations with more than 16 per cent fiber resulted in a significantly lower milk production.

An interesting experiment in this field recently has been reported by Huffman and Duncan (7). These workers used 12 cows in 15 trials to study the

effect on milk production after a part of the total digestible nutrients in alfalfa had been replaced by corn. The replacement of a part of the alfalfa hay by corn on an equal total digestible nutrient basis always resulted in an increased production of 4 per cent fat corrected milk. After the change back to an all-alfalfa ration, a definite drop in milk production occurred. Possible explanations for the increased production are discussed in the report mentioned. It is suggested that the corn grain supplies an unidentified factor or factors needed to balance alfalfa hay for milk production.

It should be noted that in all the trials reported (7), a lower level of crude fiber was fed when part of the hay was replaced by corn. For example, in trial no. 1 *b* a decrease from 29 per cent to 23 per cent fiber occurred.<sup>2</sup> In trial 2, the decrease was from 31 to 24 per cent fiber. In trial 3, the decrease in fiber was from 32 to 25 per cent and in trial 4, it was from 32 to 19 per cent. Similar results were obtained for the other trials. From the results obtained in our experiment, we believe that this reduction in the level of fiber definitely has influenced the milk production.

In a study of the optimum level of crude fiber in the feed it should be remembered that crude fiber has different chemical composition in different feeds and that its main components, cellulose, lignin and pentosans, may occur in quite different ratios (11). Therefore, it could not be expected that the optimum value for fiber in feeding experiments always will be the same in different feeds. Variations may occur according to the type of roughage used. For the type of fiber occurring in the two main feeds used in this experiment, namely, pineapple bran and Napier grass, the results mentioned in this paper may be expected, however. On the other hand, it appears possible that in a roughage such as clover hay and alfalfa, the optimum level of crude fiber may coincide with another, possibly a higher, level of the fiber.

#### SUMMARY

A feeding experiment has been carried out with different levels of crude fiber in the feed of dairy cows of Holstein-Friesian breed. Except for fiber, other nutritive factors (digestible protein and T.D.N.) were kept alike in different rations. A sufficient supply of vitamins and minerals also was provided. Four different rations were tried, namely, A with 60 lb. Napier grass, B with 40 lb. Napier grass, C with 20 lb. Napier grass daily per cow and D with no Napier grass. The balance needed of protein and T.D.N. was made up of pineapple bran, soybean oil meal, meat meal, fish meal and molasses. The crude fiber content for different rations were in percentage of dry matter as follows: A, 22.7 per cent; B, 19.6 per cent; C, 16.1 per cent; and D, 12.0 per cent. The effect of the level of crude fiber in the feed upon milk production was highly significant. With increase over 16 per cent in crude fiber content of the feed, a drop in milk production occurred, regardless of the fact that equal amounts of T.D.N. and digestible protein were fed.

The average daily yield of 4 per cent F.C.M. per cow for 12 wk. was 20.6 lb.

<sup>2</sup> Figured on the dry matter basis.

on ration A, 21.7 lb. on ration B, 23.4 lb. on ration C, and 23.8 lb. on ration D. Pulse rates per minute for cows with no roughage were 62, and increased to 69 with roughage. The statistical significance of this influence of rations fell close to the 1 per cent point.

For high milk production, the crude fiber level in the feed should not exceed 16 per cent when calculated on basis of dry matter content of the ration. This holds true for feeds such as mature Napier grass and pineapple bran as major constituents of the rations.

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## STUDIES ON KETOSIS IN DAIRY CATTLE. X. THE EFFECT OF A VITAMIN A DEFICIENCY<sup>1</sup>

J. C. SHAW

*Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park*

In earlier studies, it was observed that the blood plasma carotene and vitamin A of cows exhibiting spontaneous ketosis were quite normal, showing that the ketotic condition was not caused by a vitamin A deficiency. Likewise, these animals did not respond to massive oral doses of vitamin A (1). This latter observation was confirmed by Hayden *et al.* (2).

However, these studies did not answer the question as to whether a vitamin A deficiency in cows would produce ketosis. It appeared possible that the typical symptoms and blood picture associated with ketosis could be produced by a combination of a vitamin A deficiency and fasting immediately postpartum, since fasting during the early postpartal period usually results in a marked hypoglycemia and ketonemia (3), and the symptoms associated with a vitamin A deficiency resemble those frequently observed in ketosis. This report is believed to provide a rather conclusive answer to the above question.

### EXPERIMENTAL METHODS

The data reported herein were obtained from an experiment designed to study the influence of quality and quantity of feed upon the incidence of ketosis. In the early part of the study, it was observed that the blood plasma carotene and vitamin A values were lower than was expected; so much so, in fact, that the decision was made to continue the animals on the same diet and study the influence of a vitamin A deficiency on ketosis. The variable factors planned in the beginning were protein, fat, soluble carbohydrate and energy intake. Timothy hay (U. S. no. 2) was used because of its relatively low protein content. Raw soybeans were used as a source of protein and fat and made up 40 per cent of the concentrate ration (K-3) which was fed to all three groups for 4 mo. prepartum and to groups 1 and 2 postpartum. In addition to the soybeans, the concentrate mixture consisted of beet pulp 30 per cent, molasses 20 per cent, crushed barley 5 per cent, ground wheat 3 per cent, steamed bone meal 1 per cent, and iodized salt 1 per cent. Group 3 received concentrate ration K-1 during the postpartal period. It consisted of beet pulp 50 per cent, crushed barley 30 per cent, ground wheat 18 per cent, steamed bone meal 1 per cent, and iodized salt 1 per cent.

Morrison's feeding standards (4) were used throughout the experiment for calculating the total digestible nutrient requirements. The cows were fed rather heavily during the prepartal period to get them in a relatively fat condition. Prior to being placed on experiment, all of the cows had received liberal amounts of corn silage and a good quality of lespedeza hay in addition to a concentrate.

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TABLE 1  
*Blood plasma carotene and vitamin A of cows in groups 1, 2, and 3*

Cow	Days	Vitamin A concentrate fed prepartum	Plasma carotene			Plasma Vitamin A			Remarks
			Day of parturition	5-7 d. post-partum	10-14 d. post-partum	Day of parturition	5-7 d. post-partum	10-14 d. post-partum	
		Total I.U.	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )	
Group 1									
Becky H <sup>a</sup>				21.6	27.6		5.7	6.0	Retained placenta
Lou A			49.2	31.2	29.4	5.2	3.0	4.5	Milk fever
Dita H			27.0	33.6	24.0	5.4	3.9	3.9	
Viola J			96.0	82.2	90.0	8.1	7.5	13.2	
Av.			57.4	42.2	42.8	6.2	5.0	6.9	
Group 2									
Lively G			30.6	29.4	43.2	4.5	3.3	7.8 <sup>b</sup>	Retained placenta
Matilda H			31.8	26.4	29.4	2.7	4.8	4.5	Retained placenta
Lobelia A			47.4	47.4	48.0	4.2	4.8	5.1	Milk fever
Lizzie G			42.6	37.8	56.4	3.3	5.1	8.1	
Av.			38.1	35.3	44.3	3.7	4.5	5.9	
Group 3									
Katrina J	52	6,000,000	67.8	53.4	31.8	18.0	11.4	9.9	Milk fever
Melanie H	43	4,800,000	36.0	30.6	39.0	10.8	6.0	16.8	Milk fever
Burke H	21	2,000,000	30.6	51.0	64.2	5.7	8.4	6.0	Milk fever
Lorna A	10	1,200,000	39.6	30.6	33.6	6.6	5.4	5.4	Retained placenta
Maggie H	26	2,800,000	30.0	45.6	33.0	8.4	7.2	7.2	
Av.			40.8	42.2	40.3	9.9	7.7	9.1	

<sup>a</sup> Letter denotes breed.

<sup>b</sup> Vitamin A concentrate given per os a few days previously; value not included in average.



Beginning approximately 4 mo. prepartum, the cows were changed to timothy hay and the 40 per cent soybean ration. During the fourth and third months prepartum, the cows were fed all they would eat up to 140 per cent of requirements. During the 2-mo. dry period this was increased to 180 per cent.

In the postpartal period, the cows in group 1 received a rather high level of energy intake, whereas the cows in groups 2 and 3 were limited to approximately 50 per cent of their total digestible nutrient requirements for 3 wk. and then put on full feed as rapidly as possible. The hay was fed at the rate of 1.5 lb. per 100 lb. live weight to all cows during the prepartal period and to the cows in group 1 during the postpartal period. The cows in groups 2 and 3, which were maintained on a low level of energy intake postpartum, received 0.8 lb. of hay per 100 lb. weight during the first 3 wk. postpartum and 1.5 lb. thereafter. The concentrate mixture constituted the remainder of the total digestible nutrients. In addition, the cows in group 3 received 400,000 I. U. of a vitamin A concentrate twice per week for varying periods prepartum as indicated in table 1. The dosage was kept relatively low because of the opportunity afforded by this study to determine whether a relatively small intake of vitamin A over short periods would suffice to prevent the injury to the fetus which otherwise was certain to occur. Two additional cows were included in group 2 in the original study, but they calved before the vitamin A analyses were initiated and so are not included in this report.

Blood samples for carotene, vitamin A and glucose determinations were drawn at frequent intervals prepartum, on the day of parturition and twice per week thereafter.

A modification of the procedure of Moore (5) and of Kimble (6) was used for plasma vitamin A and carotene. Somogyi's (7) modification of the Shaffer and Somogyi method was used for blood glucose.

## RESULTS

In table 1 the plasma carotene and vitamin A values of 13 cows are shown for the period immediately postpartum. The cows in groups 1 and 2 exhibited marked vitamin A depletion, as will be observed from the plasma vitamin A values. The cows in group 3, which received the vitamin A concentrate, exhibited a somewhat higher level of plasma vitamin A during the postpartal period.

An even better estimation of the degree of vitamin A depletion of these cows may be obtained from the data presented in table 2 on their calves. All of the calves from the cows in groups 1 and 2 showed evidences, usually marked, of vitamin A deficiency. At 5 days of age, only one of the calves from these eight cows was normal in appearance, even though the calves were allowed free access to the colostrum of their dams for the first 3 days. The one calf (Viola's) which was normal in appearance at 5 days, received 500,000 I. U. of vitamin A concentrate on the day of birth. Additional evidence of the degree of vitamin A depletion in these cows was the low level of vitamin A in the colostrum, which was indicated by the relatively small increase in the plasma vitamin A of the calves by the fifth day after birth.

TABLE 2  
*Blood plasma carotene and vitamin A of calves from cows in groups 1, 2, and 3*

Dam of Calf	At birth		5 d. after birth <sup>a</sup>	
	Carotene	Vitamin A	Carotene	Vitamin A
	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )	( $\mu\text{g./100 ml.}$ )
	General condition		General condition	
	Group 1		Group 2	
Becky		Weak, eye hemorrhage		Weak, died of scours at 2 wk.
Lou	1.8	2.1		Scours
Dita	0.0	1.2		Did not recover from paralysis
Viola	1.8	6.0 <sup>b</sup>		Good
Av.	1.2	1.7		
	Group 2		Group 3	
Lively	1.2	2.4		Killed on 2nd day
Matilda				Dead at birth
Lobelia	1.2	3.6		Weak
Lizzie	2.4	2.7		Scours
Av.	1.6	2.9		
Katrina	0.6	3.0		Good
Melanie	0.6	2.4		Good
Burke	0.6	1.5		Good
Lorna	0.0	1.2		Died of bacteremia
Maggie	0.6	2.7		Good
Av.	0.5	2.2		

<sup>a</sup> All calves were allowed to remain with dams for 3 d.

<sup>b</sup> Obtained colostrum before sample was taken

<sup>c</sup> Received 500,000 I.U. of vitamin A *per os* on day of birth } not included in averages.

TABLE 3  
*Blood glucose level and general condition of cows depleted of vitamin A and on varying levels of energy intake postpartum*

Cow	Blood glucose in mg./100 ml.										Comments on symptoms of ketosis
	Days prepartum		Day of parturition	Days postpartum							
	15-13	8-6		3-4	7-8	10-11	13-14	17-18	20-21	23-24	
				Group 1							
Becky	-	43.6	52.4	34.1	43.6	39.2	36.0	43.4	44.1	45.6	Negative
Lou	39.0	39.9	57.3	29.9	38.6	37.8	33.5	42.6	38.1	40.0	Negative
Dita	-	45.1	63.5	33.6	42.1	27.9	39.4	30.5	38.6	38.6	Negative
Viola	46.3	45.6		38.1	39.4	41.9	38.6	35.4	37.5	41.9	Negative
Av.	42.7	43.6	57.7	33.9	40.9	36.7	36.9	38.0	39.6	41.5	
				Group 2							
Lively	50.0	46.4	65.6	25.7	38.1	33.8	31.1	35.1	28.1	39.4	See table 4
Mathilda	47.3	45.1	62.1	41.0	33.2	38.1	35.8	34.6	30.2	49.1	Negative
Lobelia	41.2	38.0		34.8	32.7	37.8	38.1	34.0	28.5	38.6	Negative
Lizsle	38.1	46.4	71.8	33.3	29.2	25.9	21.4	17.6	20.3	37.0	Negative
Av.	46.7	44.0	66.5	33.7	33.3	33.9	31.6	30.3	26.5	41.0	
				Group 3							
Katrina	41.9	43.5	61.8	44.6	38.3	29.2	44.6	38.1			Negative
Melanie	42.6	36.7	92.6	31.1	22.4	17.0	18.6	35.4	35.9		Negative
Burke	40.3	48.6	88.0	36.7	31.3	21.1	21.9	21.9	25.4	49.4	Negative
Lorna	41.7	47.0		36.2		31.3	27.3	34.6	33.5	38.6	Negative
Maggie	45.4	48.1	67.8	27.8	37.8	27.3	33.2	24.0	26.5	41.3	Negative
Av.	42.4	44.8	62.0	35.3	32.5	31.5	29.1	30.8	30.3	43.1	

The relatively small amount of vitamin A concentrate administered to the cows in group 3 protected four of the five calves from showing symptoms of a vitamin A deficiency at birth. The one calf in this group which showed evidence of a vitamin A deficiency was from the cow Lorna, which did not receive any vitamin A supplement until 10 days before parturition. On the other hand, the feeding of a small amount of vitamin A supplement to the cows Maggie and Burke, beginning with the 26th and 21st days prepartum, was sufficient to prevent the development of any external signs of a vitamin A deficiency in their calves.

An analysis of the hay showed that the cows were receiving approximately 35  $\mu$ g. of carotene per pound of body weight from this feed. Since the concentrate mixture supplied very little vitamin A, the cows apparently were just at or slightly below their minimum requirements for carotene intake (8). However, the very low blood plasma vitamin A levels of the cows at time of parturition and the marked symptoms of vitamin A deficiency observed in the calves at birth suggested that the vitamin A depletion of the cows was greater than could be explained on the basis of the carotene intake. It was suspected that the high proportion of soybeans in the ration was responsible inasmuch as Hilton *et al.* (9) had observed that the feeding of soybeans to cows depressed the vitamin A content of the butter obtained from these cows. A carefully controlled experiment then was conducted with calves in which it was found (11) that the feeding of soybeans did indeed exert a very marked depressing effect upon both the plasma and liver vitamin A.

The data in tables 1 and 2 show that the cows in groups 1 and 2 were depleted of vitamin A to such an extent that not only were the plasma vitamin A values of the cows extremely low, but most of the calves showed evidences of vitamin A deficiency of considerable severity at birth.

The blood glucose values and observations on possible symptoms of ketosis in these cows during the postpartal period are presented in table 3. The cows in group 1 had blood glucose levels which are in the normal range for well-fed cows during the postpartal period, on the basis of a very large volume of data (unpublished) which has been accumulated in this laboratory. Some decrease usually occurs during this period. No symptoms of ketosis were observed.

The cows in groups 2 and 3 which received a lower level of energy intake postpartum (approximately 50 per cent of requirements) exhibited a lower level of blood glucose than the cows in group 1. However, the decrease in blood glucose was of the same magnitude in the cows in group 3, which received a vitamin A supplement, as in the cows in group 2 which were depleted of vitamin A. No symptoms of ketosis were observed in any of the animals.

One of the cows (Lively) in group 2 showed marked symptoms of a vitamin A deficiency. The blood plasma carotene and vitamin A and the blood glucose values obtained on this cow are presented in some detail in table 4. It will be noted that the plasma vitamin A values were very low immediately prepartum and postpartum. The calf was completely blind and paralyzed at birth. On the fourth, fifth and sixth days following parturition, this cow exhibited night

TABLE 4  
The effect of low energy intake postpartum on the blood glucose level of a cow exhibiting a marked vitamin A deficiency

Date	Plasma carotene ( $\mu\text{g./100 ml.}$ )	Plasma vitamin A ( $\mu\text{g./100 ml.}$ )	Blood glucose ( $\text{mg./100 ml.}$ )	Per cent of T.D.N. require- ments consumed	Remarks
7/25	189.6	14.7	41.9	126	
8/8	132.0	18.0	41.6	135	
8/22	90.6	13.5	46.5	136	
8/28				163	Beginning of dry period
9/19	60.6	11.7	46.4	161	
10/14	45.6	9.9	38.3	138	
10/17	46.2	5.1	46.4	151	
10/21	27.0	5.4	44.8	91	
10/22	30.6	4.5	65.6	66	Calved, calf blind and paralyzed, cow retained placenta
10/23				55	Calf died
10/24		1.5		55	Placenta removed
10/25	30.0		25.7	42	
10/26				31	
10/27				55	
10/28	29.4	3.3	25.4	55	Cow exhibited slight incoordination and night blindness
10/29 A.M.	34.8	4.8	38.1	60	Cow exhibited marked incoordination and night blindness
P.M.					
10/30	30.6	14.1	43.5	57	1,000,000 I. U. vitamin A per os
10/31				45	1,000,000 I. U. vitamin A per os, marked incoordination
11/1	38.4	16.2	33.8	44	1,000,000 I. U. vitamin A per os, slight improvement
11/2				50	1,000,000 I. U. vitamin A per os, definite improvement
11/3				52	1,000,000 I. U. vitamin A per os, definite improvement
11/4	43.2	7.8	31.0	51	1,000,000 I. U. vitamin A per os
11/5				53	Still showed some night blindness and slight incoordination
11/6				47	1,000,000 I. U. vitamin A per os
11/7	40.8	12.6	35.1	47	Appeared almost normal
11/11	39.0	8.1	28.1	55	Put on full feed
11/13				95	
11/14	23.4	5.1	39.4	95	
11/18	30.0	6.3	42.7	104	

blindness and incoordination. On the sixth day, the incoordination was so marked that the cow had considerable difficulty maintaining her equilibrium. A careful study of the blood glucose values fails to show any evidence that the hypoglycemia of ketosis is associated with a vitamin A deficiency. On the day that the general incoordination of the animal was most severe and might have been assumed to compare with the incoordination often associated with ketosis, the blood glucose value had risen to an almost normal level of 38.1 mg. per cent. With the oral administration of large doses of vitamin A, the cow improved rapidly but the blood glucose again decreased. When the cow was put on "full feed," the blood glucose returned to normal very quickly, indicating that the low postpartal blood glucose was due to a lack of sufficient energy and not to a vitamin A deficiency.

In table 5, similar blood data are presented on a cow which was depleted of vitamin A but was maintained on a relatively high energy intake postpartum. The blood glucose level was quite normal for a cow receiving from 70 to 90 per cent of the required total digestible nutrient intake. No symptoms of ketosis were observed.

#### DISCUSSION

It is evident from the low levels of blood plasma carotene and vitamin A and the marked signs and symptoms of vitamin A deficiency in one of the cows postpartum and several of the calves at birth, that a very marked depletion of vitamin A was effected in a number of the cows during the parturient period. The fact that no symptoms of ketosis were observed and that there was no apparent relationship between vitamin A depletion and deficiency and the level of blood glucose shows that a vitamin A deficiency *per se* does not produce ketosis in cows. Superimposing fasting upon vitamin A depletion did not change this relationship. While it is preferable to determine blood ketone bodies in such studies, the fact that a hypoglycemia and typical symptoms of ketosis must exist (11) for an adequate diagnosis of ketosis makes it possible to rule out ketosis when such data do not exist. Since the degree of vitamin A depletion effected in this study seldom is observed under field conditions, it appears quite clear that the incidence of ketosis in dairy cattle is much too high to be explained on this basis even if a vitamin A deficiency did produce ketosis. It must be concluded that not only is ketosis in dairy cows, as it occurs under field conditions, not due to a vitamin A deficiency as has been reported by Patton (7) but that a vitamin A deficiency does not produce ketosis in dairy cows.

#### SUMMARY

Detailed observations were made on 13 cows which were maintained on a low carotene diet for approximately 4 mo. prepartum and 3 wk. postpartum. The vitamin A depletion of these cows was accentuated by the feeding of a concentrate ration containing 40 per cent soybeans which was later shown to exert a marked depressing action on blood plasma and liver vitamin A. Five of the cows received a vitamin A supplement prepartum which resulted in the birth of four normal-appearing calves. The other eight cows dropped calves which

TABLE 5  
*The blood glucose level of a vitamin A depleted cow on a relatively high energy intake postpartum*

Date	Plasma carotene	Plasma vitamin A	Blood glucose	Per cent of T. D. N. requirements consumed	Comments
6/27	38.4	7.2	45.1	159	
7/1	16.8	7.5	43.6	158	
7/4	27.0	5.4	63.5	41	Calved, calf exhibited general paralysis, extensive eye hemorrhage, rapid respiration
7/5	24.6	4.2	47.5	58	Calf given vitamin A supplement
7/8	33.6	6.9	33.6	70	
7/11	33.6	3.9	42.1	78	
7/15	24.6	4.5	27.9	80	Respiration of calf normal and eye more clear but paresis not improved
7/18	24.0	3.9	39.4	82	Calf was killed
7/22	27.0	4.8	30.5	89	
7/25	27.0	5.1	38.6	81	Cow exhibited no symptoms of ketosis
7/29	25.8	4.5	38.6	87	
8/1	30.0	6.0	41.6	86	
8/5	28.8	6.3	34.6	89	
8/8	33.6	7.3	47.8	88	

showed marked signs and/or symptoms of a vitamin A deficiency. The blood plasma vitamin A values of these eight cows were extremely low during the postpartal period and one cow exhibited marked symptoms of a vitamin A deficiency. Of the eight cows, four were maintained on a low level of energy intake for 3 wk. postpartum (50 per cent of requirements). The five cows receiving the vitamin A supplement prepartum were on the same low level of energy intake postpartum. In spite of the severe vitamin A depletion and deficiency produced in these cows, none showed symptoms of ketosis and the degree of hypoglycemia produced by partial fasting was as large in the case of the vitamin A-supplemented cows as in the vitamin A-depleted cows. The cows exhibiting a marked vitamin A depletion but receiving higher levels of energy intake postpartum exhibited normal levels of glucose during the postpartal period. None of the cows showed symptoms of ketosis. It is concluded that not only is spontaneous ketosis, as it is observed under field conditions, not due to a vitamin A deficiency but that a vitamin A deficiency *per se* does not produce ketosis in dairy cows in the postpartal period.

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# STUDIES ON KETOSIS IN DAIRY CATTLE. XI. LIPIDS, MINERALS AND ASCORBIC ACID IN THE BLOOD OF COWS WITH SPONTANEOUS KETOSIS<sup>1</sup>

P. SAARINEN AND J. C. SHAW

*Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park*

Rather extensive fatty infiltration and degeneration of various organs of cows with ketosis have been observed in studies carried on in this laboratory (10). This suggested that disturbances in fat metabolism may be involved. It appeared advisable, therefore, to determine whether any consistent abnormalities existed in the various blood lipids of cows with ketosis, since little information of this nature was available.

The adrenal cortex of ketotic cows always has shown extensive degeneration (11). Prior to these studies it was shown (9) that an extract of the adrenal cortex was effective in the treatment of ketosis. To obtain additional information on the possible role of the adrenals in the development of ketosis, an investigation of the level of ascorbic acid, sodium and potassium in the blood plasma also was included in this study. In addition, blood chlorides, phosphates and phosphatase activity were determined.

## EXPERIMENTAL PROCEDURE

All of the cases reported herein were field cases diagnosed as ketosis by practicing veterinarians. One of the cows studied had had ketosis in previous years and was subjected to study before and during the development of the ketotic condition. In all cases blood glucose and acetone bodies were determined as an aid in diagnosis. Some of these cases were used simultaneously for other investigations which will be reported later. The methods used are as follows: Glucose, Somogyi's (13) modification of the Shaffer and Somogyi procedure used on cadmium sulphate filtrates; acetone bodies, Barnes and Wick (1); ascorbic acid, Mindlin and Butler (5); sodium, a modification of Snell and Snell (12); potassium, Harris (2); chlorides, Whitehorn (14); phosphates, Saarinen's (6) modification of the Kuttner, Cohen and Lichtenstein procedure. This same procedure also was used in determining phosphatase activity.

The method used for blood lipids is a modification of one developed by one of the authors (7, 8). A modification of Bloor's method, developed by Katsura and associates (3, 4), was used for a comparison in developing the shorter method used in this study.

In this study instead of separating the phospholipids by precipitation, they were calculated from the difference between the amount of total lipids and the other lipids that were extracted separately. This separation is based upon the heavy hydration of phospholipids in certain pH areas where phospholipids are not extractable from aqueous emulsion with ether and petroleum ether.

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<sup>1</sup> Paper no. A269, contribution no. 2212 of the Maryland Agricultural Experiment Station.

For extraction the plasma pH is changed with a buffer solution, which liberates lipids from lipoprotein combinations so that all lipids except the phospholipids are extractable as such with low-boiling dry solvents. One method for complete fractionation of the plasma lipids based upon this procedure was published earlier by Saarinen (6, 7). In the present study instead of using this more accurate alcohol ether extraction, the total lipids were extracted from an alkaline aqueous emulsion with dry solvents after dehydrating the phospholipids with alcohol. This procedure is described in some detail.

*The extraction of the total lipids (A).* Measure 2 ml. of mixed plasma into a 25-ml. glass-stoppered graduate cylinder. Wash the pipette into the cylinder with 2 ml. of distilled water. Add 0.25 ml. of concentrated ammonium hydroxide. After mixing, add 2 ml. of redistilled alcohol (95 per cent) and mix again. Add 10 ml. of redistilled ethyl ether. Tighten the stopper with distilled water and shake vigorously by hand for 1 min. Loosen the stopper to release the pressure and allow to stand approximately 30 min. Repeat the shaking four times, allowing the mixture to stand 5 min. between shakings. Then add 10 ml. of petroleum ether and repeat the shaking four times as above. Wash the stopper with distilled water and let the cylinder stand over night or for at least 6 hr. The extract, in which 10 ml. are equivalent to 1 ml. of plasma, contains all the plasma lipids except free fatty acids but these normally are present only in negligible amounts.

*The extraction of plasma lipids other than phospholipids (B).* Measure 4 ml. of plasma into a 50-ml. glass-stoppered graduate cylinder and wash the pipette into the cylinder with 4 ml. of distilled water. Add 0.80 ml. of acetate buffer stock solution (one volume 1 N sodium hydroxide plus two volumes of 1 N acetic acid), followed by 20 ml. of redistilled ethyl ether. Stopper with moistened glass stopper and shake carefully for 30 sec. Loosen the stopper to release the pressure, stopper again and shake vigorously for 2 min. Then let stand for 20 to 30 min. After standing, shake four times, shaking for 2 min. each time and allowing to stand for 5 min. between shakings. After this extraction, 20 ml. of petroleum ether is added and the shaking is repeated four times, shaking for 1 min. each time and allowing to stand for 5 min. between shakings.

This extract, in which 10 ml. is equivalent to 1 ml. of plasma, contains all of the plasma lipids except the phospholipids.

The total amount of lipids in each extract is determined oxidometrically using the technique of Katsura *et al.*, modified as explained later. The same technique also is used for precipitating free cholesterol. The cholesterol determinations, however, are made colorimetrically, using a modification of Urbach as given by Zeiss (15).

*Oxidometric determination of lipids in extracts A and B.* Duplicate 5 ml. quantities of each extract (equivalent to 0.5 ml. plasma) are measured into acid-washed Erlenmeyer flasks of 50-ml. volume and evaporated to dryness over a boiling water bath. Traces of ammonia and acetic acid are driven off by blowing air into the flasks. Then the flasks are placed in a drying oven at

100° C. for 10 min. and air again is blown through the flasks several times. After cooling, 3 ml. of an oxidation mixture consisting of four parts of Nicloux silver dichromate-sulfuric acid solution and one part of 1 *N* aqueous potassium dichromate solution are added to each flask by means of an Ostwald pipette with a sharp tip. Two blank determinations are made simultaneously using the same procedure. The flasks are stoppered and placed over a boiling water bath for exactly 30 min. During this time, the flasks are rotated gently until all of the lipid material is dissolved, as will be indicated by a uniform adherence of the oxidation mixture on the surface of the flask. The flasks next are placed in an oven at 100 to 101° C. for exactly 1 hr., being rotated gently at 15-min. intervals and allowed to cool. After cooling, 25 ml. of distilled water are added to each flask. Just before titration, 2 ml. of 40 per cent slightly alkaline potassium iodide solution are added. The liberated iodine is titrated with a 0.05 *N* sodium thiosulfate solution, using soluble starch (1 per cent in saturated KCl solution) as indicator. The blank minus the actual titration value reveals the amount of chromic acid required for the oxidation of the lipids in extracts A and B.

*Determination of total cholesterol.* For the determination of total cholesterol, 5 ml. of each extract (A and B) are used for duplicates. If the extractions are carried out properly, the cholesterol will be extracted quantitatively in both cases. Thus, the cholesterol determination also is used to check on the quantitateness of the extractions, particularly for extraction B which requires greater care.

Five ml. of each extract is measured into a 50-ml. glass-stoppered Erlenmeyer flask, evaporated over a steam bath and dried in an oven as described previously. After cooling, 5 ml. of chloroform and 1 ml. of acetic anhydride are added and mixed. Just before warming, 1 ml. of a solution consisting of nine volumes of acetic anhydride and one volume of concentrated sulfuric acid is added and mixed by rotating. The flasks immediately are placed in the dark in a covered water bath at 38° C. for exactly 15 min. The flasks are cooled, and readings are made with a suitable photometer at a wave length of 660 *mμ*. For calculation a standard curve is established with pure cholesterol. The results are calculated on the assumption that 90 per cent of the value for blood cholesterol is due to true cholesterol (7, pp. 41 and 125).

*Ester cholesterol.* For each duplicate, 5 ml. of extract B are placed into a 50-ml. Erlenmeyer flask and evaporated to dryness over a water bath and the acetic acid is removed by passing a stream of air into the flask. The residue is redissolved with 5 ml. of acetone, followed by the addition of 2.5 ml. of 0.2 per cent digitonin solution in 95 per cent alcohol and the addition of 0.5 ml. of distilled water. After mixing, the flasks are placed on a water bath in contact with steam. The temperature of the water bath is maintained at 50 to 60° C. for 15 min., after which it gradually is increased to boiling. After evaporation the residue is dried by passing a stream of air slowly through the flask. The cholesterol esters are extracted with ether in a warm flask using three repeated extractions and 3 to 4 ml. of ether for each extraction. The portions are fil-

tered into another glass-stoppered Erlenmeyer flask through a fat-free filter and then evaporated to dryness. After cooling, 5 ml. of chloroform and 1 ml. of acetic anhydride are added and mixed. The remainder of the procedure is the same as for total cholesterol. When a limited number of determinations are being made, time may be saved by extracting the cholesterol esters from the dried residue directly with chloroform.

*Acidometric titration of non-volatile free acids in ether-petroleum ether extract.* Twenty ml. of extract B are measured into a 50-ml. Erlenmeyer flask for each duplicate and evaporated to dryness as before when the samples are prepared for oxidation of the lipids. This removes the acetic acid present in the extract. After cooling, 10 ml. of a benzene-alcohol mixture (1:1) containing 0.02 per cent phenolphthalein are added and the free acids are titrated with freshly diluted carbonate free 0.01 *N* potassium hydroxide solution using a microburette with 0.01 ml. divisions.

*Calculations.* Both total and ester cholesterol are calculated using a standard factor and the extinction value ( $L = 2 - \log G$ ) as a basis. When the amount needed for complete oxidation of total cholesterol in plasma (3.92 ml. of 0.1 *N* oxidant per 1 mg. of cholesterol) is subtracted from the titration value B (blank minus titration), the difference reveals the amount of oxidant used by the fatty acids in cholesterol and glycerol esters. This divided by the reduction constant of blood fatty acids (3.60 ml. of 0.1 *N* oxidant per 1 mg. of fatty acids) gives the amount of fatty acids in milligrams. The difference between titration values A and B shows the amount used by phospholipids. This value divided by the constant 2.82 gives the amount of phospholipids in milligrams.

If 5 ml. of extract (equivalent to 0.5 ml. plasma) are used for every determination and titrations are made with 0.05 *N* thiosulfate, the calculations of the lipid fractions would be as follows:

- (a) Total cholesterol in mg. per cent determined colorimetrically,
- (b) Ester cholesterol in mg. per cent determined colorimetrically,
- (c) Phospholipids in mg. per cent. =  $(A - B)/2.82 \times 100$ ,
- (d) Fatty acids in cholesterol and glycerol esters and as free-fatty acids in mg. per cent =  $(B - 3.92a)/3.60 \times 100$ ,
- (e) Cholesterol ester fatty acids in mg. per cent =  $0.646b$ ,
- (f) Cholesterol esters in mg. per cent =  $1.62b$  or  $0.972(b + e)$ ,
- (g) Glycerol esters + free fatty acids in mg. per cent =  $d - e$ ,
- (h) Total lipids in mg. per cent =  $a + c + d$ .

#### RESULTS

*Blood plasma lipids.* The blood plasma lipid values of cows with ketosis are presented in table 1. Because a number of the cows exhibited complications of various kinds in addition to the ketosis, an attempt was made to group the complicated and uncomplicated cases separately. On the basis of the present knowledge of ketosis in cows, such a differentiation appears advisable. Histo-pathological studies of cows with ketosis revealed that many cases exhibit some kind of complication other than that which is secondary to the ketotic condition.

TABLE 1

## Blood plasma lipids of ketotic cows

Date	Cow	Blood glucose	Blood acetone bodies	Total plasma lipids	Plasma phospholipids	Total plasma cholesterol	Plasma ester cholesterol	Plasma free cholesterol	Fatty acids in		Free acids	Days with ketosis and comments
		(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	Cholesterol esters	Glycerol esters and as free	(m. equiv./100 ml.)	
A. Apparently uncomplicated ketosis												
3/24/48	Halle	56.2	10.0	62.3	52.4	9.4	34.1	12.6				20 d., glucose adm.
3/25/48	Hall	37.5	10.9	66.6	48.2	18.4	31.3	6.0				21 d.
3/24/48	Downs I	41.9	13.4	110.9	90.5	20.4	58.8	21.2				7 d., recovering
3/24/48	Downs II	35.1		105.1	82.6	22.5	53.7	6.3				7 d., recovering
3/25/48	Flegel	39.4	20.5	98.2	78.7	19.5	51.2	3.8				6 d., recovering
7/9/48	Hermosa <sup>a</sup>	53.1	3.4	269.5	92.9	97.4	63.3	11.7			0.08	Prepartum
7/13/48	Hermosa <sup>a</sup>	50.3	5.4	226.0	80.1	67.7	43.7	21.3			0.10	Day of parturition
7/22/48	Hermosa <sup>a</sup>	40.4	6.4	238.1	100.0	56.8	28.2	36.1			0.11	Postpartum
7/28/A.M.	Hermosa <sup>a</sup>	33.3	14.4	283.2	109.2	90.5	25.7	57.8			0.09	1 d., early ketosis
7/28/P.M.	Hermosa <sup>a</sup>	23.8	15.5	254.8	79.4	86.9	30.2	56.4			0.09	1 d., early ketosis
8/2/48	Hermosa <sup>a</sup>	41.7	2.5	286.3	120.6	103.0	6.0	56.7			0.11	Recovering
8/6/48	Hermosa <sup>a</sup>	38.1	6.0	352.3	150.4	95.5	33.6	62.0			0.15	Recovering
7/28/48	Inez	20.5	31.1	245.8	66.0	101.1	17.7	61.0			0.10	4 d., glucose adm.
8/2/48	Inez <sup>a</sup>	36.7	3.3	233.4	76.6	96.7	80.4	52.2			0.12	Recovering, glucose adm.
8/6/48	Inez <sup>a</sup>	34.2	5.2	372.7	175.2	125.3	101.3	24.0			0.12	Recovering, glucose adm.
8/16/48	Beltville	22.3	45.0	291.9	104.3	137.6	103.7	33.9			0.07	1 d.
8/21/48	Beltville <sup>a</sup>	31.6	26.8	271.5	96.5	96.2	23.3	55.5			0.05	5 d.
7/8/49	King	21.6	15.0	340.7	146.1	99.6	65.2	34.4			0.19	10 d., responded to glucose adm.
Av. during ketosis				283.3	99.0	106.7	82.1	24.6	50.6	21.0	0.11	

TABLE 1 (Continued)

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies (mg. %)	Total plasma lipids (mg. %)	Plasma phospho- lipids (mg. %)	Total plasma choles- terol (mg. %)	Plasma ester- choles- terol (mg. %)	Plasma free choles- terol (mg. %)	Choles- terol esters (mg. %)	Glycerol esters and as free (mg. %)	Free acids (m. equiv./ 100 ml.)	Days with ketosis and comments
3/ 3/48	Hoffman	18.9	40.2	"	"	66.3	52.0	14.3	41.3	42.9		15 d., ruptured hypophysis
3/ 5/48	Hoffman*	28.9	30.9	"	"	72.3	55.3	17.0	45.9	44.4		17 d.
3/ 3/48	Thom*	23.5	33.7	"	"	"	"	"	"	"		9 d., glucose adm.
3/ 9/48	Thom	56.2	8.3	"	"	61.4	47.2	14.2	30.7	41.0		15 d., pneumonia
3/30/48	Cunningham	24.4	25.1	"	"	143.5	111.6	31.9	72.5	20.3		2 d., uterus inflamed
4/ 6/48	Burdette*	39.4	5.0	"	"	82.6	66.6	16.0	43.6	33.3	0.30	11 d., glucose administration
4/ 7/48	Burdette	41.2	8.1	"	"	76.6	63.7	12.9	41.4	35.8	0.28	12 d., atrophied hypophysis
2/ 7/49	Mullinix	33.7	38.9	135.5	22.7	66.1	41.0	25.1	26.7	20.0	0.21	13 d., ilium inflamed, ulcerated
3/16/49	Thomas	19.3	56.8	270.6	92.2	91.7	74.4	17.3	48.4	38.3	0.13	4 d., abomasum & duodenum slightly inflamed
3/30/49	Enterprise	22.1	46.2	314.5	92.9	125.5	102.2	23.3	66.4	29.7	0.20	16 d., excess glucose pumped in rumen
4/28/49	Sherman*	17.8	12.8	281.3	130.5	86.9	75.4	11.5	49.0	14.9	0.13	4 d., severe inflammation of abomasum & intestines
5/ 3/49	Sherman	22.9	24.0	307.6	125.3	96.7	74.9	21.8	48.6	37.0	0.18	
Average during ketosis				257.1	83.3	91.0	70.9	20.1	47.0	33.1	0.20	

\* Not included in average.

TABLE 2  
*Blood plasma lipids of cows in early and late stages of ketosis*

Date	Cow	Total plasma lipids	Plasma phospholipids	Total plasma cholesteryl	Plasma cholesteryl	Plasma free cholesteryl	Fatty acids in			Free acids
							Cholesterol esters	Glycerol esters and as free	Total	
		(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(m. equiv./100 ml.)
A. Early stage of ketosis (1-4 d.)										
7/28/48	Inez	245.8	66.0	118.8	101.1	17.7	61.0	0.0	61.0	0.10
7/28/48	Hermosa	269.0	94.3	116.7	88.5	28.0	57.1	1.0	58.0	0.09
8/16/48	Belleville 382	291.9	104.3	137.6	103.7	33.9	50.0	0.0	50.0	0.07
8/16/48	Thomas	270.6	92.2	91.7	74.4	17.3	48.4	38.3	87.7	0.13
Av.										
		269.3	89.2	116.2	91.9	24.2	54.1	9.9	64.0	0.10
B. Medium stage of ketosis (6-7 d.)										
3/24/48	Downs I			110.9	90.5	20.4	58.3	21.2	79.5	
3/24/48	Downs II			105.1	82.6	22.5	53.7	6.3	60.0	
3/25/48	Flegel			98.2	78.7	19.5	51.2	3.8	55.0	
Av.										
				104.7	83.9	20.8	54.4	10.4	64.8	
C. Late stage of ketosis (10-21 d.)										
3/25/48	Hall			66.6	48.2	18.4	31.3	6.0	36.3	
7/ 8/49	King	340.7	146.1	99.6	65.2	34.4	42.3	52.7	95.0	0.19
3/ 3/48	Hoffman			66.3	52.0	14.3	41.3	42.9	84.2	
2/ 7/49	Mullinix	135.5	22.7	66.1	41.0	25.1	26.7	20.0	46.7	0.21
Av.										
		238.1	84.4	74.7	51.6	23.1	35.4	30.4	65.6	0.20

Several cases listed as complicated would have been classified as uncomplicated if the animals had not been slaughtered. It appears, therefore, that at least some of the cows listed here in the uncomplicated group probably represent complicated cases. For example, the cow Hermosa, which appeared to be an excellent example of an early case of spontaneous ketosis, exhibited lipofibroma when posted 1 yr. later. This cow had exhibited ketosis postpartum for 3 consecutive yr. and for this reason the blood picture was followed closely immediately prepartum and postpartum. As will be observed from the blood glucose and acetone body values, blood samples were drawn from this cow before and during the development of ketosis and after recovery. In some of the cases, the blood samples were drawn after the animals had been treated with glucose so that the previous diagnosis of the veterinarian had to be used. Consequently, the blood sugar was higher and the acetone bodies lower than would have been the case before treatment. A number of these cows were slaughtered for more extensive biochemical and histo-pathological studies. Complications frequently were observed in the postmortem examinations. Blood samples frequently were drawn from the same cow in different stages of ketosis.

In computing the averages shown in table 1, the data from a single sample of blood drawn during the height of ketosis was used. Considerable variation was observed in the lipid values. All of the lipid values except the free fatty acid equivalent appeared to be somewhat lower than was to be expected. The free fatty acid equivalent was proportionally high in both complicated and uncomplicated ketosis but was observed to be highest in the cows with complicated ketosis.

These differences appeared to be due to the stage of ketosis, since the complicated cases did not respond to treatment readily and, therefore, often represented later stages of this condition. To determine whether the differences were due primarily to the stage of ketosis, some of the animals with either no complications or less severe complications were grouped into early, medium and late stages of ketosis. None of the cows grouped as early cases had exhibited signs or symptoms of ketosis for more than 4 days. Those classified as medium and late stages had exhibited signs and/or symptoms for 6 to 7 days and 10 to 21 days, respectively. As will be noted in table 2, most of the lipid fractions showed a decrease when the animals exhibited ketosis over a longer period of time. However, the free cholesterol did not appear to change appreciably, whereas the neutral fat and free fatty acid fractions usually increased.

*Blood minerals, ascorbic acid and hematocrit values.* The data on these substances are presented in table 3 for cows which exhibited ketosis of either a complicated or uncomplicated nature. The inorganic phosphorus varied widely with some values below normal. The values for blood plasma sodium, potassium and chlorine were within the normal range in most cases. The plasma ascorbic acid values also varied widely. Some of the values were low, but since the majority of the values were within the normal range it does not appear that there is any specific relationship between the blood plasma ascorbic acid and



ketosis in cows. The red cell volume usually was high, undoubtedly due to dehydration.

Because some of the inorganic phosphorus values were low, the phosphates were analysed more completely in the latter part of the study. Unfortunately, four of the five cows reported in this study (table 4) represented complicated cases. Three of the plasma acid-soluble inorganic phosphorus values were somewhat low and three of the values for serum phosphatase activity were distinctly low.

#### DISCUSSION

In general, the values for the various blood lipids, minerals and ascorbic acid of ketotic cows reported in this paper do not deviate markedly from normal values in the early stage of lactation. However, most of the blood lipid values were lower than was to be expected, with the exception of neutral fat and free fatty acids, which increased with the duration of the ketotic condition. Ordinarily, the blood plasma phospholipids and cholesterol esters decrease at parturition and then gradually increase during the second and third week postpartum. Cows with ketosis do not appear to exhibit this normal increase. In the later stages of ketosis, these values are even lower than in the early stages.

The blood mineral values of the ketotic cows were more nearly normal than the blood lipids. The fact that plasma sodium and potassium remained normal indicates that if the adrenal cortex is involved in ketosis, the factor regulating plasma sodium and potassium is not affected. Since the blood serum phosphatase activity was fairly low, it is possible that some abnormalities may exist in metabolic processes where phosphorus is involved. Several normal values for inorganic phosphorus in blood plasma indicate that a phosphorus deficiency is not associated with ketosis.

Since cows with ketosis usually exhibit inappetance, some of the alterations observed in the blood picture may be associated with fasting rather than with ketosis as such. Since little information is available on the effect of fasting in the early postpartal period upon the blood substances studied, such a study appears to be necessary before any further conclusions can be drawn.

#### SUMMARY

In a study with 18 cows diagnosed as having ketosis, an analysis was made of various blood and blood plasma substances. Plasma phospholipids and cholesterol ester fractions were somewhat low, particularly in the later stages of ketosis. Free cholesterol in the plasma was relatively normal. The amount of free ether-petroleum ether soluble non-volatile acids in plasma determined by acidometric titration was relatively high in the later stages of ketosis. The neutral fat fraction was relatively low in the early stages of ketosis and normal or high in the later stages.

Marked variations were observed in the plasma ascorbic acid values. The serum phosphatase activity was relatively low. The plasma acid-soluble phosphorus values, both inorganic and organic, were sometimes low but were normal

TABLE 3  
Hematocrit values, ascorbic acid and minerals in the blood of cows with ketosis

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies (mg. %)	Red cell volume (%)	Plasma ascorbic acid (mg. %)	Plasma Na (mg. %)	Plasma K (mg. %)	Blood Cl (mg. %)	Plasma inorg. P (mg. %)
A. Apparently uncomplicated ketosis									
4/20/46	Downey	21.3	32.2	-		345	19.4		
4/16/47	Pelagie	21.6	36.2			307	17.3		
3/24/48	Hall	56.2	10.0	36.7	0.062		22.1	307	3.0
3/25/48	Hall	37.5	10.9	35.7	0.063	248	16.8	294	3.3
3/24/48	Downs I	41.9	13.4	36.8	0.175	261	19.8	307	5.5
3/24/48	Downs II	35.1		31.4	0.062	281	22.0	314	3.0
3/25/48	Flegel	39.4	20.5	31.7	0.573	248	20.5	269	6.3
	*Av.			34.0	0.218	282	19.8	298	4.2
B. Complicated ketosis									
3/ 3/48	Hoffman	18.9	40.2		0.549			307	4.1
3/ 5/48	Hoffman	28.9	30.9		0.940			310	3.4
3/ 9/48	Thom	56.2	8.3	28.2	0.130	366	18.4	319	4.6
3/30/48	Cunningham	24.4	25.1	41.6	0.113	318	15.6	294	8.1
4/ 6/48	Burdette	39.4	5.0	30.0	0.388	252	13.2	248	6.4
4/ 7/48	Burdette	41.2	8.1	34.6	0.275	292	10.1	168	2.7
2/ 7/49	Mullinix	33.7	38.9	34.5	0.338			285	3.5
3/16/49	Thomas	19.3	56.8		0.975			300.5	2.7
3/30/49	Enterprise	22.1	46.2		0.360			275	4.1
	*Av.			34.2	0.427	312	15.2	284	

\* The value for each cow used for calculating the group averages was the average of all individual values.

TABLE 4  
Blood phosphates and phosphatase values of cows with ketosis

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies (mg. %)	Blood acid-soluble P			Plasma acid-soluble P			Phospha- tase activity (units/ 100 ml.)	Remarks
				Inorg.	Org.	Total	Inorg.	Org.	Total		
8/16/48	Beltville 328	22.3	45.0	—	—	—	6.86	0.82	7.68		Apparently uncom- plicated ketosis
2/ 7/49	Mullinix	33.7	38.9	2.50	4.30	6.80	2.71	0.83	3.54	1.57	Complicated ketosis
3/16/49	Thomas	19.3	56.8	3.68	2.45	6.12	3.54	1.31	4.85	1.05	Complicated ketosis
3/30/49	Enterprise	22.1	46.2	2.55	3.61	6.16	2.66	1.12	3.78	1.55	Complicated ketosis
4/28/49	Sherman	17.8	12.8	1.85	5.34	7.19	2.40	1.89	4.29	1.99	Complicated ketosis
Av.		—	—	2.65	3.93	6.57	3.63	1.19	4.83	1.54	

in most cases. Plasma sodium and potassium were normal and the blood chloride values also were in the normal range. Additional data on the effect of fasting and other factors secondary to ketosis are needed before the data can be properly evaluated.

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# STUDIES ON KETOSIS IN DAIRY CATTLE. XII. BLOOD LIPIDS, PHOSPHATES AND PHOSPHATASE ACTIVITY OF COWS ON DIFFERENT LEVELS OF FEED INTAKE POSTPARTUM<sup>1</sup>

P. SAARINEN AND J. C. SHAW

*Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park*

A study of cows with ketosis (1) showed some alterations in the blood plasma lipids, phosphates and serum phosphatase activity. These alterations were more distinct in cases where cows had exhibited a ketotic condition for 10 days or more. Since these cows also exhibited inanition, which usually is associated with bovine ketosis, it was difficult to decide whether the alterations were due to the ketotic condition as such or to inanition.

For purposes of comparison, a study was made of the effects of inanition during the postpartal period while the researches on ketotic cows were still in progress.

## EXPERIMENTAL PROCEDURE

At the time that this study was initiated rather extensive investigations were in progress in this laboratory relative to the effect of the quality and quantity of feed on the postpartal metabolism of the cow. The blood samples used in this study were drawn from these same animals.

For 6 mo. prior to parturition the cows all were fed rather heavily on low (10 per cent), medium (14 per cent) and high protein (23 per cent) rations. Following parturition half of the cows were fed liberally. The energy intake of the other half was limited to 35 per cent of Morrison's feeding standards for 8 to 15 days postpartum. In order to avoid repetition, the exact details relative to the feeding of these animals will be presented in a later paper together with the results of the studies for which these experiments originally were designed.

The chemical procedures used in this study are the same as those listed in the preceeding paper (1).

## RESULTS

Postpartal blood plasma lipid values from cows on different levels of protein and energy intake are presented in tables 1 and 2. The data shown in table 1 are from cows which received 70 per cent of Morrison's feeding standards for total digestible nutrients for the first week postpartum and 80 per cent during the second week postpartum. The level of protein in the ration did not exert any apparent effect upon the blood lipid values. When the postpartal plasma lipid values for the individual cows are subjected to a careful examination, it will be observed that at this level of feed intake there was a gradual increase in total lipids, phospholipids, total cholesterol, ester cholesterol and cholesterol esters, independent of the protein intake. Free cholesterol did not change appre-

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<sup>1</sup> Paper no. A270, Contribution no. 2213, of the Maryland Agricultural Experiment Station.

TABLE 1  
Blood plasma lipids of cows on high plane of nutrition postpartum

Date	Cow	Total plasma lipids (mg. %)	Plasma phospholipids (mg. %)	Total plasma cholesterol (mg. %)	Fatty acids in			Free acids (m. equiv./100 ml.)	Remarks
					Plasma ester cholesterol (mg. %)	Plasma free cholesterol (mg. %)	Cholesterol esters (mg. %)		
A. High protein ration									
8/ 4 48	Esmeralda	296.3	132.6	112.6	83.3	29.3	51.1	0.0	Prepartum
8/24 48	Esmeralda			103.7	79.9	23.8			Prepartum
8/27 48	Esmeralda	240.6	79.7	104.4	87.4	16.7	56.8	13.2	1 d. postpartum
9/17 48	Esmeralda	363.4	134.7	154.3	135.6	18.7	74.4	0.0	22 d. postpartum
1/ 3 49	Ruby	302.2		111.6	96.2	15.4			16 d. postpartum
1/17 49	Ruby	498.1	263.8	191.0	124.3	66.7	43.3	0.0	30 d. postpartum
Av. 16 d. postpartum (Ruby)									
		302.2		111.6	96.2	15.7			0.20
B. Medium protein ration									
5/12 48	Bonita	250.0	76.6	82.3				91.1	0.10
5/19 48	Bonita	243.8	80.9	87.4				75.5	0.08
5/20 48	Bonita			90.4				111.0	0.11
5/26 48	Bonita	262.9	82.3	58.6				122.0	0.15
6/ 2 48	Bonita	311.0	109.9	67.2				133.9	0.10
7/22 48	Faith	336.2	148.9	122.9	84.5	38.4	54.9	9.5	0.05
7/28 48	Faith	306.2	122.7	129.1	100.3	28.8	54.4	0.0	0.04
8/11 48	Faith	281.8	78.9	114.0	96.7	17.3	62.9	26.0	0.18
8/21 48	Faith	361.7	120.4	159.6	131.8	27.8	81.7	0.0	0.08
Av. 13-14 d. postpartum									
		336.4	115.2	113.4	131.8	27.8	81.7	0.0	0.09
C. Low protein ration									
8/11 48	Acacia	210.3	37.6	95.5	76.1	19.4	49.4	27.8	0.06
8/27 48	Acacia	295.2	116.3	120.0	105.8	14.2	58.9	0.0	0.10
9/ 3 48	Acacia	334.0	123.4	120.1	114.0	16.1	74.1	6.4	0.06
9/17 48	Acacia	355.4	90.1	184.8	174.5	10.3	80.5	0.0	0.06
1/ 3 49	Pomona	243.5		85.7	68.4	17.3			0.15
1/17 49	Pomona	375.9	117.5	171.8	133.2	38.6	86.6	0.0	
Av. 12 d. postpartum (Acacia)									
		334.0	123.4	130.1	114.0	16.1	74.1	6.4	0.06
Av. of all three groups									
12-16 d. postpartum		324.2	119.8	118.4	114.0	19.8	77.9	3.2	0.13

TABLE 2  
*Blood plasma lipids of cows on low plane of nutrition postpartum*

Date	Cow	Total plasma lipids	Plasma phospho lipids	Total plasma cholest-erol	Plasma ester cholest-erol	Plasma free cholest-erol	Fatty acids in		Free acids	Remarks
							Cholest-erol esters	Glycerol esters and as free		
		(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(m. equiv./100 ml.)	
A. High protein ration										
6/ 9 48	Martha	286.7	87.9	93.8					0.09	Prepartum
6/22 48	Martha	238.7	74.5	89.8	68.7	21.1	45.6	28.8	0.14	9 d. postpartum
6/25 48	Martha	255.2	92.2	86.9	69.7	17.2	45.3	30.8	0.12	12 d. postpartum
6/28 48	Martha	247.9	86.5	80.9	68.7	12.2	60.3	20.2	0.13	15 d. postpartum
7/28 48	Beth	211.7	59.6	93.8	74.2	19.6	48.2	10.1	0.06	Prepartum
8/19 48	Beth	199.8	56.7	77.0	66.1	10.9	43.0	23.1	0.12	Prepartum
8/23 48	Beth	212.6	81.5	73.9	63.0	10.9	41.0	16.2	0.10	3 d. postpartum
8/31 48	Beth	239.4	74.5	93.8	81.1	12.7	52.7	18.4	0.18	11 d. postpartum
1/ 3 49	Lizzie	148.1		88.1	69.2	18.9			0.13	11 d. postpartum
3/10 49	Elinor	250.0	56.7	102.2	88.1	14.1	57.3	33.8	0.05	Prepartum
4/ 1 49	Elinor	233.1	71.6	68.7	55.9	12.8	85.0	7.8	0.19	10 d. postpartum
B. Medium protein ration										
Av. 8-15 d. postpartum <sup>a</sup>		216.9	76.8	84.1	68.8	15.3	62.7	17.6	0.15	
5/ 5 48	Valencia	198.4	66.7	72.2			59.5		0.13	Day of parturition
5/ 8 48	Valencia	233.2	54.6	88.0			90.6		0.18	3 d. postpartum
5/12 48	Valencia	236.4	73.8	69.8			92.8		0.11	7 d. postpartum
5/14 48	Valencia	250.9	90.8	90.6			69.5		0.13	9 d. postpartum
5/17 48	Valencia	263.4	85.1	92.2			86.1		0.13	12 d. postpartum
5/19 48	Valencia	241.4	80.9	95.5			65.0		0.12	14 d. postpartum
7/13 48	Adventuress	260.5	76.6	91.7	77.5	14.2	50.3	41.9	0.07	Prepartum
7/22 48	Adventuress	208.4	59.6	72.7	64.2	8.5	41.7	34.4	0.06	Prepartum
7/28 48	Adventuress	174.7	60.3	77.5	59.3	18.2	38.5	58.7	0.04	Prepartum
8/ 2 48	Adventuress	179.2	71.6	67.0	57.2	9.8	37.2	3.4	0.08	1 d. postpartum
8/ 9 48	Adventuress	205.8	56.0	78.7	62.8	15.9	40.8	30.3	0.14	8 d. postpartum
8/13 48	Adventuress	231.0	57.4	89.8	67.7	22.1	57.5	26.3	0.20	12 d. postpartum
8/16 48	Adventuress	259.2	75.2	100.1	82.6	17.5	53.7	30.2	0.19	15 d. postpartum

TABLE 2 (continued)  
*Blood plasma lipids of cows on high plane of nutrition postpartum*

Date	Cow	Total plasma lipids (mg. %)	Plasma phospho-lipids (mg. %)	Total plasma cholest-erol (mg. %)	Plasma ester cholest-erol (mg. %)	Plasma free cholest-erol (mg. %)	Fatty acids in		Free acids (m. equiv./100 ml.)	Remarks
							Cholest-erol esters	Glycerol esters and as free		
9/13 48	Bounty	248.8	73.0	94.1	82.8	11.3	53.8	27.9	0.14	1 d. postpartum
9/17 48	Bounty	297.8	120.6	117.2	110.2	7.0	60.0	0.0	0.10	5 d. postpartum
9/23 48	Bounty	309.4	111.3	116.4	98.2	18.2	63.8	17.9	0.15	11 d. postpartum
Av. 8-15 d. postpartum*		264.4	86.6	99.6	84.6	18.4	53.9	26.2	0.15	
C. Low protein ration										
6/ 9 48	Bunny	279.0	87.2	101.5	89.8	11.1	56.9	90.3	0.10	Prepartum
6/22 48	Bunny	312.0	127.0	100.9	71.3	17.3	46.3	27.5	0.08	Prepartum
6/28 48	Bunny	239.9	83.0	88.6	71.3	16.8	35.0	22.0	0.09	1 d. postpartum
7/ 1 48	Bunny	254.6	129.8	89.8	73.0	40.2	30.7	0.0	0.14	4 d. postpartum
7/ 6 48	Bunny	254.1	120.6	87.4	47.2	15.4	37.0	15.4	0.10	9 d. postpartum
7/ 9 48	Bunny	210.1	61.0	70.8	57.0	13.8	37.0	41.3	0.15	12 d. postpartum
7/12 48	Bunny	248.1	98.6	70.6	69.7	0.9	45.3	33.6	0.09	15 d. postpartum
8/ 4 48	Remembrance	276.2	114.9	99.1	91.7	7.4	59.6	2.6	0.06	Prepartum
8/31 48	Remembrance	229.6	71.6	100.8	75.4	25.4	49.0	8.2	0.10	1 d. postpartum
9/ 3 48	Remembrance	259.0	92.2	92.4	79.7	12.7	51.8	22.6	0.13	4 d. postpartum
9/ 7 48	Remembrance	214.2	72.3	70.2	57.7	12.5	37.5	34.2	0.17	8 d. postpartum
2/18 49	Melanie	322.3	85.8	117.1	93.2	18.9	98.2	21.2	0.05	Prepartum
2/25 49	Melanie	285.7	24.1	146.6	130.3	16.3	84.6	30.4	0.08	Prepartum
3/ 2 49	Melanie	313.1	87.2	110.9	91.2	19.7	59.3	55.7	0.10	Prepartum
3/21 49	Melanie	222.7	47.5	71.3	63.3	8.0	41.1	62.8	0.15	9 d. postpartum
3/23 49	Melanie	226.6	53.9	106.6	86.9	19.7	56.5	9.6	0.20	11 d. postpartum
Av. 8-15 d. postpartum		225.4	72.1	78.5	63.6	14.9	41.3	33.5	0.14	
Av. of all three 8-15 d. postpartum		235.6	78.5	87.4	72.3	16.2	52.6	25.8	0.15	

\* The value for each cow used for calculating the group averages was the average of all values between 8 and 15 d. postpartum.



TABLE 3  
*Blood plasma lipids in early and late stages of ketosis and the effect of postpartum fasting on plasma lipids*

	No. of cows	Total plasma lipids	Plasma phospholipids	Total plasma cholesterol	Plasma ester cholesterol	Plasma free cholesterol	Fatty acids in			Free acids
							Cholesterol esters	Glycerol esters and as free	Total	
		(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(m. equiv./100 ml.)
Early stage of ketosis (1-4 d.) <sup>a</sup> . . .	2-4	269.3	89.2	116.2	91.9	24.2	54.1	9.9	64.0	0.10
Later stage of ketosis from 6th day on . . .	2-6	238.1	84.4	87.6	65.4	22.1	43.5	21.8	65.3	0.20
Cows on high plane of nutrition postpartum	4	324.2	119.8	118.4	114.0	19.8	77.9	3.2	81.1	0.13
Cows on low plane of nutrition postpartum	10	235.6	78.5	87.4	72.3	16.2	52.6	25.8	78.4	0.15
Cow starved for 8 d. postpartum . . . . .	1	184.8	24.5	88.1	61.3	26.8	39.6	32.6	72.2	0.44

<sup>a</sup> Data from (1)

ciably. There are insufficient values for free acids to draw any precise conclusions. However, it will be noted that the free acids are lower before than after parturition. Also, when the values for the cows on a low plane of nutrition postpartum (table 2) are compared to those on a higher plane of nutrition (table 1), the free acids usually were higher after the cows had been on a low energy intake for several days.

TABLE 4  
*Plasma phosphate and phosphatase values of cows on high and low planes of nutrition postpartum*

Date	Cow	Plasma acid-soluble P			Phosphatase activity	Remarks
		Inorg.	Org.	Total acid-soluble		
		(mg. %)	(mg. %)	(mg. %)	(units/100 ml.)	
A. Cows on high plane of nutrition postpartum						
9/ 3 48	Esmeralda	5.91	2.12	8.03		High protein feeding
9/17 48	Esmeralda	6.42	0.95	7.37		High protein feeding
12/ 3 48	Peggy	2.94	0.71	3.65		Medium protein feeding
12/ 4 48	Peggy	3.59	1.05	4.64		Medium protein feeding
2/11 49	Peggy				3.07	Medium protein feeding
8/27 48	Acacia	3.91	1.58	5.49		Low protein feeding
9/ 3 48	Acacia	6.76	1.82	8.58		Low protein feeding
Av. of individual averages		4.92	1.37	6.29	3.07	
B. Cows on low plane of nutrition postpartum						
8/25 48	Beth	6.03	0.77	6.80		High protein feeding
8/31 48	Beth	4.20	0.86	5.06		High protein feeding
3/28 49	Elinor				3.07	High protein feeding
4/ 1 49	Elinor	4.58	0.92	5.50	1.92	High protein feeding
9/17 48	Bounty	4.46	1.18	5.64		Medium protein feeding
9/20 48	Bounty	4.56	0.69	5.25		Medium protein feeding
8/31 48	Remembrance	4.88	1.92	6.80		Low protein feeding
9/ 9 48	Remembrance	4.05	0.72	4.77		Low protein feeding
3/21 49	Melanie	1.87	0.43	2.30	1.58	Low protein feeding
3/23 49	Melanie	3.59	0.83	4.32	1.41	Low protein feeding
3/28 49	Melanie				2.22	Low protein feeding
Av. of individual averages		4.28	0.92	5.20	2.11	

The data in table 2 are from cows which received 35 per cent of Morrison's feeding standards for total digestible nutrients for from 8 to 15 days postpartum. The difference in the protein intake did not appear to influence the blood lipids. However, several of the lipid fractions were altered by the low energy intake. Whereas several of the plasma lipid fractions of the cows on the higher energy intake (table 1) increased markedly in the early postpartal period, these same fractions either decreased or exhibited but a slight increase when the energy intake was maintained at a low level (table 2). This picture is similar to the results obtained with cows in early and late stages of ketosis (1). For purposes of comparison, a summary is given in table 3 of the data in table 1 and 2 together

with a summary of the data previously presented on ketotic cows (1). Cows receiving 70 to 80 per cent of Morrison's feeding standards for total digestible nutrients presented a blood plasma lipid picture very similar to that of cows in the early stages of ketosis. The cows on the lower plane of nutrition postpartum showed a blood plasma lipid picture very similar to that found in cows which had exhibited ketosis for some period of time. Apparently, the alterations in blood plasma lipids observed in cows with ketosis are due to the inanition which is associated with ketosis, rather than to ketosis as such. For purposes of comparison a Guernsey cow was fasted completely for 8 days beginning 3 wk. postpartum. At the beginning of the fasting period the blood plasma lipid picture was as follows: total lipids, 278.4 mg. per cent; plasma phospholipids, 95.0 mg. per cent; cholesterol-glycerol ester plus free fatty acid fraction, 183.4 mg. per cent; and free acids, 0.12 milliequivalents per 100 ml. of plasma. These values appear to be about normal for a cow in this stage of lactation. The plasma lipid values after 8 days of fasting are shown in table 3. Complete fasting produced blood lipid changes of a similar nature but of a greater magnitude than was noticed in ketotic cows.

In table 4, some data are presented on blood plasma acid soluble phosphates of cows on high and low levels of energy intake postpartum. Fasting appeared to have little or no effect on the plasma acid soluble phosphates. These data are quite similar to those observed in cows with ketosis (1).

Values are presented on serum phosphatase activity of two cows which were partially fasted postpartum. These values were somewhat below normal during the fasting period, which indicates that the low values observed in ketotic cows (1) may have been due to inanition.

#### SUMMARY

To determine whether some of the alterations previously observed in the blood lipids and phosphatase values of cows with ketosis are due to ketosis *per se* or secondarily to the inanition associated with ketosis, 16 cows were used in a study of the effect of different levels of protein and energy intake postpartum. The postpartal plasma lipid values of cows receiving 70 to 80 per cent of their total digestible nutrient requirements were similar to those of cows in the early stages of ketosis. The postpartal plasma lipid values of cows receiving only 35 per cent of their total digestible nutrient requirements were similar to those of cows in the later stages of ketosis. Complete fasting for 8 days produced alterations of the same nature but of greater magnitude.

#### ACKNOWLEDGMENT

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# STUDIES ON KETOSIS IN DAIRY CATTLE. XIII. LIPIDS AND ASCORBIC ACID IN THE LIVER AND ADRENALS OF COWS WITH SPONTANEOUS AND FASTING KETOSIS<sup>1</sup>

P. SAARINEN AND J. C. SHAW

*Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park*

The few reports available (1, 2, 3, 6, 9) relative to the condition of the liver of cows and ewes with ketosis deal primarily with the pathology of this organ. In these reports a fatty liver always has been observed to be a part of the ketotic syndrome so that it has been assumed rather generally that the livers of ketotic ruminants always are fatty. It also has been shown by Groenewald *et al.* (1) with ewes and by Shaw *et al.* (8) on cows, that the adrenals tend to be fatty. The adrenal also was implicated when an extract from it was found to promote recovery of cows with ketosis (Shaw, 7).

It was deemed advisable to conduct further studies to determine whether these abnormalities are associated with the early development of ketosis or are secondary to the inanition associated with ketosis.

## EXPERIMENTAL PROCEDURE

Studies were conducted on cows exhibiting spontaneous ketosis and on cows which had been fed at either a medium or low plane of nutrition postpartum. The feeding and management of the experimental cows was discussed rather briefly in a previous report (5). The blood glucose and acetone bodies were determined in all cases but will be reported elsewhere in connection with other studies. The methods used were similar to those discussed in a previous communication with the exception that the lipids were extracted from the ground tissue by repeated extraction with a warm alcohol-ether mixture (2:1) and purified by resolving in petroleum ether. Ascorbic acid was extracted from macerated tissue with 2.5 per cent metaphosphoric acid. Liver samples were obtained by biopsy and also after slaughter.

## RESULTS

Liver lipid and ascorbic acid values of cows with both uncomplicated and complicated ketosis are presented in table 1. When compared with normal cows in mid-lactation, the total liver fat values will be observed to be high in most cases. The total cholesterol of the liver of the ketotic cows was much higher than that of normal cows in mid-lactation, the increase being due mainly to the ester cholesterol fraction. The free cholesterol fraction which represents the main form of cholesterol in the liver of normal cows is proportionally low in cows with ketosis. The ascorbic acid values vary widely, with some of the values being relatively low.

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TABLE 1  
Total fat, cholesterol and ascorbic acid in the liver of cows with "spontaneous" ketosis

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies	Total liver fat (%)	Liver cholesterol			Liver ascorbic acid (mg. %)	Days with ketosis and comments
					Ester cholesterol	Free cholesterol	Total cholesterol		
		(mg. %)	(mg. %)	(%)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	
					A. Apparently uncomplicated ketosis				
3/25/48	Hall	37.5	10.9	22.3	393.4	99.9	493.3	36.3	21 d., glucose administered
7/28/48	Inez	29.5	31.1	9.1	136.6	77.6	214.2		4 d., glucose administered
7/28/48	Hernosa	23.8	15.5	4.0	167.3	0.0	167.3		1 d., early ketosis
8/17/48	Belleville (328)	22.3	45.0	6.9	213.0	82.6	295.6		2 d., early ketosis
7/ 9/49	King	21.6	15.0	23.3	329.0	113.6	442.6		11 d., glucose administered
	Av.			13.1	247.9	74.7	322.6	36.3	
					B. Complicated ketosis				
3/ 9/48	Hoffman	23.9	30.9	23.6	305.2	115.6	420.8	42.4	21 d., ruptured hypophysis
3/12/48	Thom	56.2	8.3	27.0	491.0	81.8	572.8	10.1	21 d., pneumonia
3/30/48	Cunningham	24.4	25.1	13.1	93.4	203.3	296.7	30.7	2 d., uterus inflamed
4/ 8/48	Burdette	41.2	8.1	11.2	72.4	193.2	265.6	29.1	13 d., atrophied hypophysis
2/ 7/49	Mullinix	33.7	38.9	16.7	308.0	43.0	351.0	16.2	13 d., ilium inflamed, ulcerated
3/17/49	Thomas	19.3	56.8	9.5	195.7	61.7	257.4	16.9	5 d., abomasum and duodenum slightly inflamed
5/ 3/49	Sherman	22.9	24.0	8.4	289.0	24.8	313.8	25.3	9 d., severe inflammation of abomasum and intestines
	Av.			15.8	250.7	103.3	354.0	24.4	
					C. Normal cows in mid-lactation				
6/29/48	Roma	-	...	4.0	57.7	137.3	195.0		
7/ 6/48	Roma	-	-	2.8	37.4	180.8	218.2		
6/29/48	Belladonna	-	-	2.2	25.7	111.3	137.0		
7/ 6/48	Belladonna	-	-	1.8	35.8	108.4	144.2		
	Av.			2.7	39.2	134.5	173.6		

The data on the liver lipids were grouped according to the stage of ketosis as shown in table 2. These data show, contrary to the general belief, that liver

TABLE 2

*Total fat and cholesterol in the livers of cows in early and in late stages of ketosis*

Date	Cow	Total liver fat	Liver cholesterol		
			Ester cholesterol	Free cholesterol	Total cholesterol
		(%)	(mg. %)	(mg. %)	(mg. %)
A. Early stage of ketosis (from 1-5 d.)					
7/28/48	Inez	9.1	136.6	77.6	214.2
7/28/48	Hermosa	4.0	167.3	0.0	167.3
8/17/48	Beltsville (328)	6.9	213.0	82.6	295.6
3/17/49	Thomas	9.5	195.7	61.7	257.4
	Av.	7.4	178.2	55.5	233.8
B. Later stage of ketosis (from 11-21 d.)					
3/25/48	Hall	22.3	393.4	99.9	493.3
7/ 8/49	King	23.3	329.0	113.6	442.6
3/ 9/48	Hoffman	23.6	305.2	115.6	420.8
2/ 7/49	Mullinix	16.7	308.0	43.0	351.0
	Av.	21.5	333.9	93.0	426.9

fat may be normal or only slightly increased in the early stages of ketosis. The data on the liver fat of the cow, Hermosa, are of particular interest. A sample of liver was taken by liver biopsy on the first day that any signs or symptoms of ketosis were observed. The blood glucose showed a sharp drop on this day and the first increase was noticed in the blood acetone bodies. The liver fat was only 4 per cent which is quite low for this stage of lactation. The total liver fat of the cow Beltsville 328, which also was a very early case of ketosis, was only 6.9 per cent. In later stages of ketosis the total liver fat always was high. The increase in liver cholesterol, especially in the ester fraction, clearly is associated with the stage of ketosis, since there was a marked elevation in the later stages of ketosis.

The postpartal liver lipid values of normal cows on different levels of protein and energy intake during the postpartal period are shown in tables 3 and 4. For purposes of comparison, prepartal values also were determined on these cows. Table 4 represents cows on a low level of energy intake postpartum and table 3 includes cows on a higher level of energy intake during the postpartal period.

As will be observed in table 3, the postpartal liver fat and cholesterol values were somewhat higher than before parturition or in mid-lactation (table 1). At this higher level of energy intake, the level of protein intake did not appear to influence the liver lipids.

The data in table 4 are in rather sharp contrast to those in table 3. A low level of energy intake postpartum increased the total liver fat markedly as well as the liver cholesterol, especially the ester cholesterol fraction. In case of fast-

TABLE 3

*Fat and cholesterol in the livers of cows on a high plane (70-80%) of nutrition postpartum*

Date	Cow	Total liver	Liver cholesterol			Remarks
			Ester cholesterol	Free cholesterol	Total cholesterol	
		(%)	(mg. %)	(mg. %)	(mg. %)	
<b>A. High protein feeding</b>						
8/20/48	Esmeralda	2.3	93.5			Prepartum
9/ 9/48	Esmeralda	5.5	182.8	64.4	247.2	14 d. postpartum
12/18/48	Ruby	6.8	102.1	356.8	458.9	Day of parturition
1/ 3/49	Ruby	5.4	36.3	253.9	290.2	16 d. postpartum
4/ 6/49	Anxiety	4.1	.....			Prepartum
4/20/49	Anxiety	6.7	.....			12 d. postpartum
4/29/49	Virginia	9.7	.....			13 d. postpartum
5/ 4/49	Canary	3.1	.....		212.5	Prepartum
6/ 3/49	Canary	4.2	392.7	259.3	652.0	14 d. postpartum
Av. of individual av. (12-16 d. postpartum)		5.8	203.7	192.5	376.1	
<b>B. Medium protein feeding</b>						
5/14/48	Bonita	3.5	....		247.0	Prepartum
5/27/48	Bonita	9.2	....		227.0	7 d. postpartum
6/ 3/48	Bonita	5.8			276.0	10 d. postpartum
8/ 7/48	Faith	8.1	93.2	168.2	261.4	Day of parturition
8/20/48	Faith	6.5	237.8			13 d. postpartum
Av. of individual av. (10-13 d. postpartum)		6.7	237.8		276.0	
<b>C. Low protein feeding</b>						
8/13/48	Acacia	4.5	121.0	58.9	179.9	Prepartum
9/ 3/48	Acacia	6.0				12 d. postpartum
12/21/48	Pomona	5.1	103.2	138.1	241.3	Prepartum
1/13/49	Pomona	5.9	67.0	371.0	438.0	13 d. postpartum
4/ 6/49	Charm	3.8	.....			Day of parturition
4/20/49	Charm	8.2	.....			14 d. postpartum
4/ 8/49	Hilda	3.9	.....			Prepartum
4/29/49	Hilda	7.1			416.7	12 d. postpartum
Av. of individual av. (12-14 d. postpartum)		6.8	67.0	371.0	427.2	
Av. of all groups (10-16 d. postpartum)		6.7	169.5	281.8	359.8	

ing, the level of protein also appears to have exerted an effect, the total liver fat and the ester cholesterol usually being higher when the protein intake was limited.

For purposes of comparison the liver lipid values in early and in later stages of ketosis are presented in table 5, together with those of cows on low and higher levels of energy intake postpartum. The total liver fat and ester cholesterol values are quite similar when these values for cows in the early stages of ketosis are compared to the postpartal values of cows on a 70 to 80 per cent plane of nutrition. Likewise, these values were increased both in the later stages of ketosis and on the lower level of nutrition postpartum. In the later stages of ketosis the total liver fat, total liver cholesterol and ester cholesterol

TABLE 4  
Total fat and cholesterol in the liver of cows with fasting ketosis

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies (mg. %)	Total liver (%)	Liver cholesterol			Remarks and days fasted postpartum
					Ester cholesterol (mg. %)	Free cholesterol (mg. %)	Total cholesterol (mg. %)	
A. High protein feeding								
6/ 9/48	Martha	50.8	4.6	5.1	-	-	-	Prepartum
6/20/48	Martha	41.0	9.4	4.2	-	-	-	7 d. postpartum
8/13/48	Beth	47.8	3.0	4.1	121.3	62.9	184.2	Prepartum
8/24/48	Beth	37.8	7.4	9.6	168.4	66.7	235.1	4 d. postpartum
8/31/48	Beth	16.8	24.6	15.8	196.7	7.4	204.1	11 d. postpartum
12/21/48	Lizzie	48.4	3.7	4.7	36.3	163.4	199.7	Prepartum
1/ 3/49	Lizzie	24.1	18.0	11.1	126.7	99.0	225.7	11 d. postpartum
3/11/49	Elinor	46.8	3.0	4.1	112.8	140.9	253.7	Prepartum
4/ 1/49	Elinor	12.2	23.7	8.3	123.8	7.0	130.8	10 d. postpartum
4/29/49	Barbara	49.5	-	3.0	98.7	63.9	162.6	Prepartum
5/13/49	Barbara	11.9	-	13.9	88.6	107.1	195.7	Day of parturition
Av. of individual av. (10-11 d. postpartum)								
				11.7	149.1	37.8	186.9	
B. Medium protein feeding								
4/27/48	Valencia	44.3	3.8	4.3	-	-	-	Prepartum
5/14/48	Valencia	26.5	9.3	16.4	164.2	-	248.0	9 d. postpartum
5/19/48	Valencia	26.8	8.2	13.4	-	-	516.0	14 d. postpartum
5/20/48	Valencia	-	-	12.5	-	-	265.0	15 d. postpartum
7/29/48	Adventress	42.7	1.9	2.6	22.5	94.4	265.8	Prepartum
8/10/48	Adventress	20.2	25.3	15.3	201.0	76.4	116.9	9 d. postpartum
8/16/48	Adventress	33.9	20.5	17.3	183.7	99.7	277.4	15 d. postpartum
9/ 9/48	Bounty	50.5	2.7	3.1	59.6	136.5	283.4	Prepartum
9/13/48	Bounty	46.1	1.5	4.7	198.1	32.1	196.1	1 d. postpartum
9/23/48	Bounty	20.7	23.6	-	129.6	179.6	230.2	11 d. postpartum
9/25/48	Bounty	41.4	10.6	6.6	66.8	163.7	309.2	Full-fed
Av. of individual av. (9-15 d. postpartum)								
				15.2	160.9	133.8	230.5	
							312.8	



TABLE 4 (continued)

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies (mg. %)	Total liver (%)	Liver cholesterol			Remarks and days fasted postpartum
					Ester cholesterol (mg. %)	Free cholesterol (mg. %)	Total cholesterol (mg. %)	
					C. Low protein feeding			
6/ 9/48	Bunny	47.1	3.1	4.0			209.4	Prepartum
7/ 9/48	Bunny	35.8	17.2	16.2	168.8	116.1	284.9	12 d. postpartum
7/12/48	Bunny	33.4	17.5	12.7	232.0	28.7	260.7	15 d. postpartum
8/20/48	Remembrance	43.5	1.0	1.8	106.3		...	Prepartum
8/31/48	Remembrance	47.4	4.1	6.5	77.6	166.5	244.1	1 d. postpartum
9/ 9/48	Remembrance	27.2	21.6	21.4	232.7	98.7	331.4	10 d. postpartum
2/25/49	Melanie	51.6	3.4	4.7	53.6	146.0	199.6	Prepartum
3/23/49	Melanie	25.1	15.6	28.4	434.5	80.9	515.4	11 d. postpartum
4/20/49	Melanie	35.6		12.4	325.1	160.8	485.9	Full-fed
Av. of individual av.								
				21.4	239.2	84.0	373.2	(10-15 d. postpartum)
Av. of all groups								
				16.1	199.7	85.2	290.9	(9-15 d. postpartum)

in the liver were somewhat higher than was the case when the cows were on a 30 per cent level of nutrition postpartum.

Table 5 also includes liver lipid values for a cow fasted for 8 days beginning 3 wk. postpartum. At the beginning of the fasting period, the total liver fat was 5.5 per cent. After 8 days of fasting it had increased to 36.6 per cent. Both free and total cholesterol values were of about the same magnitude as in the later stages of ketosis. The ester cholesterol showed about the same increase as was observed in the later stages of ketosis. Free cholesterol had decreased to about the same extent as was observed in ketosis and as the result of lowered energy intake postpartum. The increase in liver fat was in all cases due almost entirely to an increase in the neutral fat fraction.

TABLE 5

*Comparison of liver lipid values in early and later stage of ketosis and on low and higher levels of energy intake for 9-16 days postpartum*

	No. of animals	Total liver fat	Liver cholesterol		
			Ester cholesterol	Free cholesterol	Total cholesterol
		(%)	(mg. %)	(mg. %)	(mg. %)
Cows with early ketosis (1-5 d.)	4	7.4	178.2	55.5	233.8
Cows with late ketosis (11-21 d.)	4	21.5	333.9	98.0	426.9
Cows on higher plane of nutrition postpartum	11	6.4	169.5	281.8	359.8
Cows on lower plane of nutrition postpartum	9	16.1	199.7	85.2	290.9
Cows fasted for 8 d. postpartum	1	36.6	287.7	95.8	383.5

A similar comparison to that presented in tables 1 to 5 was made on the adrenals. These data are shown in table 6. The total fat of the adrenals of ketotic cows was higher than that of normal cows and usually higher than that of cows partially fasted postpartum. Four such values in the later stages of ketosis were higher than that of a cow in the earlier stage. The ester cholesterol showed the opposite picture. Partial fasting appeared to increase the adrenal fat to some extent, but not as much as in the case of some of the cows with ketosis. Complete fasting for 8 days resulted in a total adrenal fat of 13.8 per cent, which is higher than most of the observations made on ketotic cows. The free cholesterol value was higher than any previously observed. The adrenals of cows with either spontaneous ketosis or fasting ketosis exhibited low ester cholesterol and high free cholesterol.

The adrenal ascorbic acid values of cows with ketosis were somewhat low (table 6); however, the adrenal ascorbic acid content of a normal cow on a low energy intake postpartum also was low and the adrenal ascorbic acid content of the cow fasted completely was the lowest observed.

#### DISCUSSION

These data are believed to be the first to demonstrate that the liver lipids of cows in the early stages of spontaneous ketosis may be relatively normal and that the extremely fatty livers of these cows are associated with the later stages

TABLE 6  
Total fat, cholesterol and ascorbic acid in the adrenals of cows with "spontaneous" ketosis and of cows on varying levels of energy intake postpartum

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies (mg. %)	Total adrenal fat (%)	Adrenal cholesterol			Adrenal ascorbic acid (mg. %)	Comments
					Ester cholesterol (mg. %)	Free cholesterol (mg. %)	Total cholesterol (mg. %)		
A. Apparently uncomplicated ketosis									
3/25/48	Hall	37.5	10.9	5.9	54.5	204.9	259.4	66.6	Glucose administered
7/9/48	King	21.6	15.0	8.6	164.8	222.8	387.6		Glucose administered
Av.				7.3	109.7	213.9	323.5	66.6	
B. Complicated ketosis									
3/9/48	Hoffman	28.9	30.9	7.6	170.5	217.3	387.8	64.4	Glucose administered
3/12/48	Thom	56.2	8.3	4.9	56.0	211.1	267.1	106.0	
3/30/48	Cunningham	24.4	25.1	14.0	62.3	257.6	319.9	110.0	
4/8/48	Burdette	41.2	8.1	11.2	70.3	309.1	379.4	62.2	
2/7/49	Mullinix	33.7	38.9	6.9	160.3	143.7	304.0	85.9	
3/17/49	Thomas	19.3	56.8	4.8	46.8	232.6	279.4	90.4	
Av.				8.2	94.3	228.6	322.9	86.5	
C. Early stage of ketosis (5 d.)									
2/17/49	Thomas			4.8	46.8	232.6	279.4	90.4	
D. Later stage of ketosis (10-21 d.)									
3/25/48	Hall			5.9	54.5	204.9	259.4	66.6	
7/8/48	King			8.6	164.8	222.8	387.6	64.4	
3/9/48	Hoffman			7.6	170.5	217.3	387.8	85.9	
2/7/49	Mullinix			6.9	160.3	143.7	304.0	90.4	
Av.				7.3	137.5	197.2	334.7	72.3	

TABLE 6—(Continued)  
*Total fat, cholesterol and ascorbic acid in the adrenals of cows with "spontaneous" ketosis and of cows on varying levels of energy intake postpartum*

Date	Cow	Blood glucose (mg. %)	Blood acetone bodies (mg. %)	Total adrenal fat (%)	Adrenal cholesterol			Adrenal ascorbic acid (mg. %)	Comments
					Ester cholesterol (mg. %)	Free cholesterol (mg. %)	Total cholesterol (mg. %)		
E. Normal cows									
4/ 8/48	Eskay 1			3.4	94.0	223.4	317.4	149.5	Mid-lactation
12/21/49	Eskay 2			3.4	178.7	135.8	314.5		Later stage of lactation
12/21/49	Eskay 3			3.2	297.2	95.8	393.0		Later stage of lactation
12/21/49	Eskay 4			3.1	208.0	123.3	331.3		Later stage of lactation
12/21/49	Eskay 5			2.7	159.0	200.7	359.7		Later stage of lactation
12/21/49	Eskay 6			3.1	226.5	98.9	325.3		Later stage of lactation
	Av.			3.2	193.9	146.3	340.2	149.5	
F. Cows on low plane of nutrition postpartum									
5/20/48	Valencia			6.2	50.2	277.5	327.7	92.0	Medium protein feeding
7/12/48	Bunny			4.4	190.3	98.7	289.0		Low protein feeding
	Av.			5.3	120.3	188.1	308.4	92.0	
G. Cow fasted for 8 d. postpartum									
2/ 1/50	Lizzie			13.8	82.9	502.4	585.3	56.7	

of ketosis. It appears, therefore, that a fatty liver is not a primary cause of ketosis in cows. The data on the effect of fasting during the postpartal period suggest that the fatty liver associated with ketosis in cows is due to inanition. The same is true regarding the increase in the total cholesterol and ester cholesterol in the liver. The fatty adrenals observed in ketotic cows also appears to be due, mainly, to fasting. In both liver and adrenals the increase in fat was due, primarily, to neutral fat. However, fasting resulted in a decrease of free cholesterol and an increase of ester cholesterol in the liver and an increase in free cholesterol of the adrenals. The adrenals of the cows with ketosis were enlarged and flabby but contained more dry matter than was found in the adrenals of normal cows. The dry matter content of the adrenals was determined in the last three cases of ketosis studied and varied from 22.3 to 28.9 per cent. The adrenals taken from five normal cows showed a lower and rather constant dry matter content varying only from 20.5 to 21.3 per cent. The adrenal gland of the cow fasted completely for 8 days contained 24.6 per cent dry matter and 13.8 per cent fat but was smaller and firmer than the adrenals of the cows with ketosis. The results of a histological study of these glands will be reported elsewhere.

#### CONCLUSIONS

Liver lipids were determined on 12 cows with spontaneous ketosis, 24 normal cows on various levels of protein and energy intake postpartum and on two normal cows in mid-lactation. Similar studies were made on the adrenals of nine cows with ketosis, two normal cows which were partially fasted postpartum, one normal cow which was fasted completely for 8 days postpartum and one normal cow in mid-lactation.

The results show that, contrary to general belief, the fat content of the liver often presents normal postpartal values in the early stages of ketosis. The fatty liver appears in the later stages of ketosis. This effect was reproduced by fasting postpartum, and indicates that the fatty liver associated with spontaneous ketosis is due for the most part to inanition. The total cholesterol, and especially the ester cholesterol fraction followed the same pattern. It is concluded that a fatty liver is not a predisposing factor in the development of most cases of spontaneous ketosis.

Postpartum-fasted cows which received a low protein ration, both before and after parturition, exhibited livers with a higher fat content than cows on a high protein ration.

The high fat content of the adrenals of ketotic cows also was reproduced by fasting. The free cholesterol increased and the ester cholesterol decreased in both fasting and spontaneous ketosis, which is opposite to the change observed in livers.

Some of the ascorbic acid values of both the liver and adrenals of cows with ketosis were somewhat low. This also was reproduced by fasting.

The flabbiness of the adrenals observed in ketotic cows was not reproduced by fasting and could not be explained on the basis of the water or fat content of these glands.

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## STUDIES OF HEATED MILK. II. ACETOL AND RELATED COMPOUNDS<sup>1</sup>

DAVID G. KEENEY,<sup>2</sup> STUART PATTON AND DONALD V. JOSEPHSON

*Department of Dairy Husbandry, The Pennsylvania Agricultural Experiment Station,  
State College*

It has been reported previously that the heating of skim milk evidences the formation of carbonyl compounds, and further, that these compounds can be removed from the milk for further study by such procedures as steam distillation or ether extraction (11). Since these compounds are heat generated in the milk, and, as such, represent end products of the chemical changes taking place, it was considered expedient to accomplish their identification insofar as possible.

### EXPERIMENTAL

**Acetol.** Forty pounds of condensed skim milk (29 per cent total solids) were autoclaved in a sealed, stainless steel can at 122° C. for 3 hr. and then cooled to room temperature. The contents of the can were filtered, the curd discarded and the brown whey-filtrate retained for steam distillation. Steam distillation was accomplished using conventional apparatus, mineral oil being employed as an anti-foaming agent. The distillate was collected for a period of 45 min. after which time the distillate was transferred to a separatory funnel and extracted five times with an equal volume of ethyl ether. The ether layers were combined, set aside for other investigations and attention given to the extracted distillate. It was found to give the following reactions: an orange precipitate with 2,4-dinitrophenylhydrazine reagent, iodoform with iodine-potassium iodide reagent and a brownish-red color when submitted to the nitroprusside test, this color becoming a stable greenish-blue upon acidification of the reaction mixture.

Sufficient of the 2,4-dinitrophenylhydrazone (2,4-DNPH) for purification and recrystallization was prepared by adding 0.5 g. of the reagent in 10 ml. of concentrated H<sub>2</sub>SO<sub>4</sub> to 750 ml. of extracted distillate. After 4 hr. the precipitated reaction product was recovered by filtration. It was found to be slightly soluble in alcohol and to give a purple color when treated with dilute alcoholic sodium hydroxide. Strain (13), among others, has indicated that the bis 2,4-DNPH's of glyoxal and diacetyl exhibit a dark blue color on treatment with alcoholic alkali. The product was recrystallized from nitrobenzene and after two such treatments no melting point increase could be effected, the final melting point being 296–297° C. with decomposition.

A search of the literature revealed methylglyoxal as forming a 2,4-DNPH of this melting point, the reported values being 296–297, 297, 298 (2) and 299–300 (7). The carbon, hydrogen and nitrogen analyses of the derivative from

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<sup>2</sup> Present address: M and R Dietetic Laboratories, Inc., Columbus, Ohio.

extracted distillate were observed to compare well with those of the bis 2,4-DNPH of methylglyoxal: Calculated for  $C_{15}H_{12}O_8N_8$ , carbon 41.67 per cent, hydrogen 2.80 per cent and nitrogen 25.92 per cent; found, carbon 41.82 per cent, hydrogen 3.21 per cent, nitrogen 25.67 per cent.

The above data, together with the qualitative reactions given by the extracted distillate, strongly suggested that acetol or methylglyoxal might be the compound in question. Both of these compounds give the same 2,4-DNPH and similar results with many qualitative tests (12). The authentic 2,4-DNPH of methylglyoxal was made and observed to melt at 296–297° C., both alone and when intimately mixed with the derivative prepared from the heated milk distillate.

With the identity of the derivative established, it remained to ascertain whether the parent compound was acetol or methylglyoxal. Both the steam distillate of heated milk and the ethyl ether extractable material from heated milk gave positive results in the test for acetol developed by Baudisch and Deuel (1). This test depends upon the reaction of acetol with *o*-aminobenzaldehyde to give 3-hydroxyquinaldine, which melts at 265° C. and gives a brilliant blue fluorescence in dilute aqueous solution when exposed to ultraviolet light. This reaction is not given by methylglyoxal (1). In conducting the tests for acetol in heated milk, the 3-hydroxyquinaldine was isolated and identified by melting point, as well as by its blue fluorescence.

*Acetaldehyde.* For the most part, the carbonyl compounds of heated milk appear to be present in quantities too small to permit direct study. However, the conversion of these compounds into their 2,4-DNPH's afforded a practical approach to the problem, providing some suitable method could be developed for separating and purifying the mixed derivatives. With this aim in mind, chromatographic separation of the derivatives prepared from the ether extract of heated milk was attempted. These experiments were largely unsuccessful. However, it seems salient, in view of recent findings (10), to report the isolation of the 2,4-DNPH of acetaldehyde in one instance. The pure crystalline derivative was found to melt at 166–167° C. and to give elemental analyses which compared very well with those calculated for the 2,4-DNPH of acetaldehyde, melting point, 168° C. (9). The mixed melting point of the unknown and an authentic derivative showed no depression (166–167° C.).

*Acetic acid.* During certain phases of this investigation, the presence of acetic acid in ethyl ether extract residues from heated milk was fairly obvious by odor alone. Since the presence of this compound in heated milk does not appear to have been reported previously, a concerted effort was made to confirm the fact. Acetic acid was isolated and identified both from the steam distillate and the ether extract of heated milk. For the sake of brevity, only an account of the steam distillate isolation will be presented.

Concentration of the acids present in 2 l. of steam distillate, collected from 50 lb. of autoclaved skim milk (29 per cent total solids), prepared as previously described, was effected as follows: The pH of the distillate was adjusted to the phenolphthalein end point with sodium carbonate, after which treatment the distillate was extracted four times with equal volumes of ethyl ether. The ex-



tracted distillate was concentrated under vacuum to a volume of 75 to 100 ml. This concentrate was acidified to liberate the acids and then extracted five times with 100-ml. volumes of ethyl ether. The combined ether layers were dried and the solvent removed by evaporation in a water bath at 50° C. The pungently acidic residue (3 g.) was distilled and yielded three fractions boiling as follows: 99.5–100, 100–110 and 110–115° C. Distillation was stopped at 115° C., since it had been noted previously that the temperature rose very rapidly thereafter and that decomposition of the residue occurred. The two lower-boiling fractions were observed, by qualitative tests, to contain appreciable quantities of formic acid. The fraction boiling 110–115° C., of about 0.5 g. in weight, had a strong, distinct odor of acetic acid and contained no formic acid, as evidenced by a very slow reaction with  $\text{KMnO}_4$  reagent. A *p*-nitrobenzyl ester derivative, melting point 77–78° C., was prepared from this fraction. This derivative showed no depression in melting point on admixture with *p*-nitrobenzylacetate, the accepted melting point for which is 78° C. (8). In additional experiments, the presence of acetic acid was further confirmed by observation of refractive index,  $n_D^{25}$  1.372; the preparation of the *p*-bromphenacyl ester, melting point 84–85° C. (8) and conversion of the acetic acid to ethyl acetate.

*Control experiments.* Ten pounds of condensed skim milk, processed in the same manner as the condensed milks used for autoclaving, were steam distilled for a period of 45 min. Periodic tests on the distillate indicated an absence of carbonyl compounds during the first 25 min., after which time a very slight positive reaction with 2,4-dinitrophenylhydrazine reagent could be noted. The absence of these compounds at the start of the treatment would appear to be the critical point. With the autoclaved milks, the first 5 to 10 min. of distillation gave the greatest yield of carbonyl compounds, after which time they were continuously evolved at a lower rate. The distillate from the non-autoclaved milk exhibited no distinct acidity. Thus, the chemical differences in the steam distillates of autoclaved and non-autoclaved condensed skim milks were amply demonstrated for the purposes of this investigation.

#### DISCUSSION

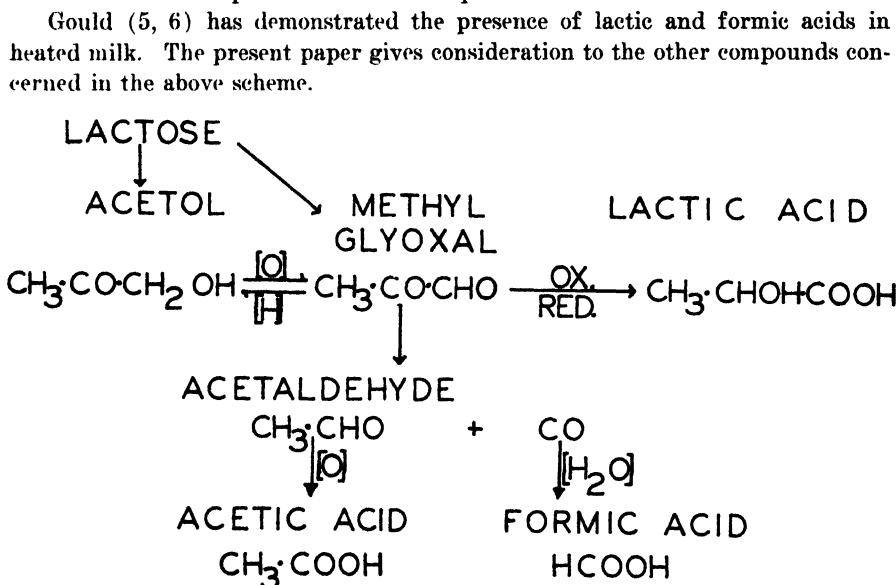
The results of this investigation have established acetol as one of the compounds produced in milk by heat. However, the possibility that methylglyoxal was copresent has not been precluded by this study.

From earlier literature in the field of carbohydrate chemistry (1, 4), it might have been anticipated that acetol or methylglyoxal is formed in milk as a result of heating. Merely heating aqueous sugar solutions has been reported to produce small quantities of the above compounds (3). However, the matter of distinguishing between acetol and methylglyoxal when present at great dilution poses a difficult problem. This subject has been reviewed thoroughly and greatly clarified in a recent paper by Sattler and Zerban (12).

Although data in the literature concerning the physical and chemical properties of methylglyoxal are scarce, observations concerning the acetol-methylglyoxal relationship in these experiments indicated that the latter is present in traces,

if at all. Methylglyoxal is reported to commence boiling at 72° C. (7). No such compound was detected in this study. On the other hand, the ether extract fraction giving a positive Baudisch and Deuel test for acetol, was observed to be relatively stable and to boil in the vicinity of 140° C. These properties are in keeping with those of acetol. Sattler and Zerban (12) have observed a ratio of roughly 500 parts of acetol to one part of methylglyoxal in their study of this matter. It seems unlikely that methylglyoxal, an unstable, highly reactive compound, would exist in free form, in heated milk (122° C.) for any appreciable length of time.

The mechanism of acetol and methylglyoxal formation from sugars has been reviewed (3, 12). While the interrelationship of the compounds reported in this paper is a matter of conjecture, the following scheme is presented as accounting in a logical manner for the formation, in part at least, of certain of these and other compounds known to be produced in autoclaved milk.



The presence of acetaldehyde in heated milk has not been firmly established in this investigation, since it was isolated only once in the form of its 2,4-DNPH derivative. However, in view of recent findings by Mohammad *et al.* (10), it seems worthy to note the presumptive presence of this compound in heated milk. The aforementioned group has observed that the rate of browning of protein-acetaldehyde systems is about 35 times as fast as that observed with protein-glucose systems under comparable conditions.

#### SUMMARY AND CONCLUSIONS

This study has demonstrated the presence of acetol and acetic acid in autoclaved condensed skim milk. The results of adequate control experiments preclude that these compounds are present in significant amounts in unheated

milk. Presumptive but not conclusive evidence that acetaldehyde is formed during the heating of milk also was obtained.

The relationship of acetol to methylglyoxal and certain other compounds known to be present in heated milk is discussed.

A wide variety of carbonyl compounds were observed to be formed during the prolonged heat treatment of milk. These compounds, for the most part, have not yet been identified.

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## THYROPROTEIN FOR LACTATING COWS IN MID-SUMMER

K. E. GARDNER AND T. W. MILLEN<sup>1</sup>

*Department of Dairy Science, University of Illinois, Urbana*

Since thyroxin stimulates the body metabolic rate, the advisability of feeding thyroprotein to milking cows under hot weather conditions in Louisiana was questioned by Seath *et al.* (12). At environmental temperatures of 93° F. thyroprotein-fed cows showed greater temperature elevation than the controls. Seath noted large body weight losses and attempts to prevent these losses by heavier feeding were only partly successful. Weight losses by cows receiving thyroprotein have been reported by several workers (2, 6, 11, 12). Moore (6) indicated in preliminary observations that approximately 25 per cent additional T.D.N. above Morrison's requirement was needed to maintain body weight and milk flow. The Morrison requirement level to which Moore refers is that "recommended for good cows under usual conditions" (7). Thomas (15) reported from the same laboratory that thyroprotein did not change significantly the gross efficiency for milk production, or the net efficiency when cows were receiving it and were fed at the same level of T.D.N. intake as the controls. However, Thorbek *et al.* (16) in Denmark found that feeding iodized casein to cows increased their heat production 30 per cent and raised the consumption of energy nutrients for milk production by 65 per cent. Increased pulse rates resulting from thyroprotein feeding have been shown by Reineke and Turner (9), as well as others, and increased respiratory rates were reported by Blaxter (2). These physiological reactions of increased respiratory rate, pulse rate and body temperature appear to indicate an increase in heat production and greater effort by the organism to eliminate heat. This extra work occasioned by feeding the compound must result in a reduced gross efficiency of energy utilization unless there are compensatory effects from thyroprotein feeding which increase the net efficiency for milk production.

The purpose of this study was to obtain a more definite measure of the efficiency of feed utilization when thyroprotein was fed in hot weather. In addition to this, it was felt desirable to obtain further knowledge concerning the lactation response and the physiological effects of feeding thyroprotein at several different levels under Mid-west summer conditions.

### EXPERIMENTAL

At Urbana, Ill., in the summer of 1947, mean daily temperatures by months were: May, 60° F.; June, 71° F.; July, 76° F.; and August, 82.8° F. In

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<sup>1</sup> Now at Wallace High-Line Hatchery, Des Moines, Iowa.

August, the maximum daily temperature exceeded 90° F. on 23 days and 95° F. on 10 days. A double reversal and a continuous trial were conducted to study the effect of thyroprotein feeding upon milk production under such conditions.

*Double reversal trial.* The double reversal trial, consisting of three 5-wk. periods, was conducted using four cows, one each of the Ayrshire, Brown Swiss, Guernsey and Jersey breeds. The first experimental period began on June 7. Two cows received thyroprotein and two cows acted as controls during each experimental period. Thyroprotein<sup>2</sup> was fed in the concentrate mixture daily at the rate of 1.5 g. per 100 lb. body weight. The cows had been in milk from 4 to 8 mo. and none was pregnant over 8 wk. at the start of the trial. Good quality, first-cutting alfalfa hay was fed at the rate of 2 lb. per 100 lb. body weight or as near that amount as appetites would permit. During the warmest days it was found that hay lost some of its palatability when cows slobbered on it and consumption was improved by more frequent feeding. This hay analyzed 6.38 per cent moisture, 5.84 per cent ash, 1.82 per cent fat, 34.38 per cent fiber and 12.50 per cent total protein. Using Morrison's (8) coefficients of digestibility, the T.D.N. value obtained was 52.6 per cent.

The basal concentrate mixture consisted of 335 lb. ground shelled corn, 285 lb. ground oats, 150 lb. wheat bran, 50 lb. dried brewers' grains, 50 lb. soybean oil meal, 100 lb. linseed meal, 15 lb. steamed bone meal and 15 lb. salt. This mixture contained approximately 73.0 per cent T.D.N. on the basis of Morrison's tables of average composition and digestible nutrients and analyzed 10.22 per cent moisture, 4.91 per cent ash, 4.06 per cent fat, 7.68 per cent crude fiber and 14.56 per cent total protein.

During the first experimental period, the feeding of concentrates to both controls and thyroprotein-fed cows was regulated to provide, along with the hay fed, the amount of nutrients required in the Morrison standard "minimum allowance advised" (8). The T.D.N. requirement for maintenance was calculated on the basis of the weights obtained at the end of the week averaged with the previous weekend weight to give a theoretical mid-week weight. Since the cows fed thyroprotein lost weight rapidly at this rate of feeding, an attempt was made to prevent the loss in the second and third periods by increased concentrate feeding. Accurate records of feed intake were kept.

Cows were stabled during the day and were turned out into a dry lot for the night. Cows were milked by combine milking machine twice daily. All animals were fed in individual, covered mangers and were bedded on wood shavings. The cows were weighed at the same hour on 3 successive days at the beginning of the trial and at the end of each period. They were weighed on 2 successive days at the end of each week. Babcock tests were run weekly on composite samples prepared by taking an aliquot of each milking. Milk was weighed at each milking and milk yields were converted to a 4 per cent fat-corrected milk basis (3).

Blood analyses for serum calcium, phosphorus and magnesium, as well as

<sup>2</sup> The thyroprotein used was "Protamone," furnished by The Cerophyl Laboratories, Inc., Kansas City, Mo.

for blood sugar, were run in the standardization period and late in each experimental period. At least once each week in mid-afternoon, pulse rates and respiratory rates were taken. Blood pressure readings were made on the coccygeal arteries, using a broad cuff sphygmomanometer according to Harshbarger (4). The deflections of the sphygmomanometer needle provided an excellent method of measuring pulse rates. Rectal temperatures were taken twice weekly in mid-afternoon with a clinical thermometer inserted 4 in. into the rectum.

In this reversal type of experiment, the thyroprotein undoubtedly produced carry-over effects of indeterminate length in subsequent periods, not only upon production but upon the efficiency of feed utilization and upon general physiology. As a result, a continuous trial was desirable.

*Continuous trial.* Eight Holstein cows were placed on a continuous trial beginning on June 28. The cows were divided into three groups. Two animals were used as controls, three received thyroprotein at the rate of 1.33 g. per 100 lb. body weight and three received thyroprotein at the rate of 2.00 g. per 100 lb. body weight. The trial was divided into a 3-wk. preliminary period, an 8-wk. continuous thyroprotein feeding period and a 3-wk. final period. In the final period, two of the thyroprotein-fed cows continued to receive either the 1.33 or 2.00 g. rate, two cows were reduced one-half and two were removed entirely from thyroprotein feeding.

The hay and the concentrate mixture fed were the same as for the reversal trial and all other management and experimental procedures were identical to those described for the double reversal trial.

## RESULTS

*Double reversal trial.* A 32 per cent over-all increase in fat-corrected milk was observed for the four cows receiving thyroprotein at the rate of 1.5 g. daily per 100 lb. body weight. The production per cow averaged by 5-wk. periods is given in table 1 and shows a yield of 752.1 lb. F.C.M. during the periods of thyroprotein feeding, compared with 568.7 lb. while receiving the basal ration alone. During the first 5-wk. period of thyroprotein feeding, all cows showed body weight losses ranging from 26 to 128 lb., although receiving T.D.N. at 99 to 123 per cent of Morrison's minimum requirement. The smallest weight loss was for the cow receiving 123 per cent of her calculated T.D.N. requirement. On the basal mixture alone, the cows gained weight when fed at 105 to 124 per cent of calculated T.D.N. requirement. This difference in weight gain indicated a lowered efficiency of nutrient utilization when thyroprotein was fed.

In order to determine the per cent efficiency of the utilization of T.D.N. available for maintenance and production, the formula given at the bottom of table 1 was developed. Since the energy involved in weight loss became available for maintenance and production along with that in the feed consumed, such weight loss was converted to T.D.N. and added to the T.D.N. of the feed. In the case of body gain, that much T.D.N. became unavailable for maintenance and production and hence was deducted from the T.D.N. consumed. Factors

for converting weight gain or loss into T.D.N. are not considered highly accurate but for comparative purposes may serve as aids. The factors used in this study were those developed by Knott *et al.* (5) and are shown at the bottom of table 1. One pound body gain is considered equivalent to 3.53 lb. T.D.N., while a 1-lb. body loss is considered equivalent to 2.73 lb. T.D.N.

All four cows were relatively less efficient in their use of available T.D.N. during periods of thyroprotein feeding than during the periods when only the basal concentrate mixture was fed. The efficiency of T.D.N. utilization averaged 69 per cent for the thyroprotein periods and 104 per cent during the basal periods. This means that some 31 per cent of the T.D.N. available for main-

TABLE 1

*Production response and efficiency of energy utilization in a 15-wk. double reversal trial with thyroprotein fed at the rate of 1.5 g. per 100 lb. body weight*

Cow no.	Breed	Ration	Av. F.C.M. yield per period <sup>a</sup>	Total T.D.N. requirement <sup>b</sup>	Total T.D.N. consumption	Weight change in T.D.N. <sup>c</sup>	Efficiency of use of available T.D.N. <sup>d</sup>
			(lb.)	(lb.)	(lb.)	(lb.)	(%)
845	A	Thyro	1085.8 <sup>e</sup>	1312	1425	-247	78.5
		Basal	908.5	603	645	+173	127.8
901	BS	Thyro	309.8 <sup>e</sup>	843	1068	-396	57.6
		Basal	182.1	378	472	+18	83.3
926	G	Thyro	799.7	500	578	-120	71.7
		Basal	558.2 <sup>e</sup>	859	938	+71	99.1
M-59	J	Thyro	813.0	472	577	-71	72.8
		Basal	626.0 <sup>e</sup>	832	941	+177	108.8
Av.		Thyro	752.1	781.6	911.8	-208.5	69.77
		Basal	568.7	667.8	748.7	+109.5	104.47

<sup>a</sup> Fat-corrected milk (3).

<sup>b</sup> Morrison's minimum requirement (8).

<sup>c</sup> Body weight changes were converted to T.D.N., using 1 lb. gain equivalent to 3.53 lb. T.D.N. and 1 lb. loss equivalent to 2.73 lb. T.D.N. (5).

<sup>d</sup> Efficiency of utilization of available T.D.N. determined using the following formula:

T.D.N. requirement for maintenance and production

$$\frac{\text{T.D.N. available (consumed T.D.N. + T.D.N. equivalent of weight loss)}}{\text{or (consumed T.D.N. - T.D.N. equivalent of weight gain)}} \times 100 = \% \text{ efficiency of utilization of available T.D.N.}$$

<sup>e</sup> Average of two 35-d. periods.

tenance and production cannot be accounted for by weight gains or increased milk flow. Presumably, such energy has been dissipated in the production of body heat and in the energy required to eliminate that portion of the heat which must be removed to maintain proper body temperature. This loss of energy approximates Thorbek's (16) value of 30 per cent increased heat production resulting from the feeding of iodized casein.

Thomas (15) indicates no difference in caloric efficiency between control cows and thyroprotein-fed cows; but, he does show a greater efficiency when his figures are corrected for body weight gain and maintenance.

The studies of respiratory rates and heart rates assist in explaining the efficiency loss. During the warmest 5-wk. period, when the environmental temperature averaged 88° F. at the time rectal temperatures were taken, the thyro-

protein cows averaged 59 respirations per minute as compared to 42 for the two controls. The respiratory rates of the two groups showed little difference during the first and last periods when environmental temperatures averaged 70 and 76° F. During the warm middle experimental period, the thyroprotein-fed cows had a pulse rate of 97 compared to 75 for the cows receiving the basal ration. In the first period the heart rate of the experimental cows averaged 16 higher, but in the final period, when environmental temperatures were lower, there was very little difference.

Decreases in serum calcium and serum magnesium were noted during the middle of the summer for the four cows on the double reversal trial. The levels of both minerals again increased in September. This cannot be attributed to the thyroprotein feeding, but may indicate a seasonal variation. An

TABLE 2

*Production response and efficiency of energy utilization in an 8-wk. continuous trial with thyroprotein fed to Holstein cows at rates of 1.33 and 2.00 g. per 100 lb. body weight*

Cow no.	Ration	Total F.C.M. yield <sup>a</sup>	Yield change from preliminary period	Total T.D.N. requirement <sup>b</sup>	Total T.D.N. consumption	Weight change in T.D.N. <sup>c</sup>	Efficiency of use of available T.D.N. <sup>d</sup>	
							Entire 8 wk.	Second 4 wk.
		(lb.)	(%)	(lb.)	(lb.)	(lb.)	(%)	(%)
878	Basal	2096	+ 4.2	1146	1275	+ 169	103.6	95.1
M53	Basal	862	- 27.1	797	1066	+ 212	93.3	81.5
	Av.	1479	- 7.9	972	1171	+ 191	99.2	89.2
879	Thyro	1486	+ 22.6	923	1264	- 238	61.5	59.5
986	at 1.33	2037	+ 17.2	1095	1234	- 96	82.3	95.4
M14	g./100#	2421	+ 2.5	1264	1509	- 279	70.7	81.6
	Av.	1981	+ 11.9	1094	1336	- 204	71.0	77.3
925	Thyro	2617	+ 19.9	1273	1414	- 489	66.9	71.7
959	at 2.00	1781	+ 24.6	1026	1356	- 268	63.2	64.6
M16	g./100#	2397	+ 23.9	1153	1499	- 319	63.4	61.7
	Av.	2265	+ 22.5	1151	1423	- 359	64.6	66.0

See table 1 for footnotes.

analysis of variance according to Snedecor (13) showed this mid-summer effect was statistically significant at the 1 per cent level of probability. Rusoff (10) observed no significant difference in his studies of seasonal effects upon blood levels of either mineral, although there was a significant monthly variation. Thyroprotein feeding increased blood sugar 4 to 31 per cent over the basal ration, while serum phosphorus apparently was not affected.

*Continuous trial.* In the second trial the two Holstein control cows during a period of 8 wk. dropped 7.9 per cent in F.C.M. below the preliminary period as shown in table 2. At the start of the trial, cow M53 was in her 22nd week of pregnancy, which accounts in part for her relatively rapid decline in yield. The three Holstein cows receiving 1.33 g. thyroprotein per 100 lb. body weight increased 11.9 per cent in F.C.M., while the three Holsteins receiving 2.00 g. thyroprotein per 100 lb. body weight produced 22.5 per cent more F.C.M. than



in the preliminary period. These production data indicate a greater lactation response from feeding the higher level of thyroprotein.

Body weight losses again were quite large for the cows receiving thyroprotein. The body weights reached their lowest point and stabilized after 5 wk. of thyroprotein feeding. The average loss at this time was 87 lb. for the three cows on the lower rate of thyroprotein feeding and 132 lb. on the higher rate. All cows were fed well above their calculated T.D.N. requirement, as table 2 shows. This surplus of T.D.N. amounted to 22 and 19 per cent for the lower and higher rates of thyroprotein addition.

The efficiency of utilization of the available T.D.N. was studied in the same manner as explained in the double reversal trial. Again, the cows on the basal ration were much more efficient in their use of T.D.N., and 99.2 per cent of their T.D.N. intake could be accounted for in live weight gain, production and maintenance. In the group of cows receiving 1.33 g. of thyroprotein per 100 lb. body weight, the efficiency of T.D.N. utilization was 71.0 per cent while the cows receiving the 2-g. level of thyroprotein showed only 64.6 per cent efficiency. Possibly at least some of the cows began to adjust for this augmented total thyroxin supply during the second 4 wk. of the trial. Four of the six cows which received thyroprotein showed relatively higher efficiency in T.D.N. utilization during the last half of the study than for the 8 wk. as a whole, as shown in table 2.

After the 8-wk. study was completed, two of the experimental cows, M14 and 925, were continued on their original level of thyroprotein feeding for a final 3-wk. period. These cows showed efficiencies of 105 and 112 per cent. Cows 879 and M16 were reduced one-half in rate of thyroprotein feeding for this final period and showed efficiencies of 70 and 91 per cent.

The last pair of thyroprotein cows, 986 and 959, were taken off thyroprotein and, as expected, they dropped rapidly in milk yield. They gained extremely rapidly in body weight, their total gains for the 21 days amounting to 79 lb. for 986 and 119 lb. for 959. The apparent efficiency of the body weight gains would indicate that cow 986 required only 1.75 lb. T.D.N. and cow 959 only 1.13 lb. T.D.N. for each pound of gain. Gains of this magnitude are very unusual, but were observed by Andrews and Bullard (1) following the partial thyroidectomy of steers. Thomas and Moore (14) have hypothesized that the thyroid gland may function at a subnormal rate for 140 or more days following the cessation of thyroprotein feeding. If this is true, partial thyroidectomy and removal of cows from thyroprotein feeding both produced temporary hypothyroid conditions with similar results. This suggests that decreased metabolic rate may have been partially responsible for the rate and efficiency of gain noted in these two cows following withdrawal of thyroprotein from their ration. Dairy cattle studies of the tissue changes accompanying the rapid weight losses and gains in thyroprotein feeding work are needed.

The respiratory rates for the three groups were similar in the preliminary period, but during the total 8-wk. experimental period, the controls averaged 45 respirations per minute while the cows fed at the rate of 1.33 and 2.00 g. thyro-

protein respired 67 and 77 times per minute, respectively, in counts made in mid-afternoon.

Body temperatures were identical for the three groups at the start. During the 8-wk. experimental period, the controls averaged 102.0° F., while the lower and the higher rates of thyroprotein treatment averaged 102.7 and 103.4° F., respectively. Body temperatures were no higher for hot, humid days than for hot, dry days. Extended periods of hot weather affected the body temperatures particularly. Average body temperatures when the environmental temperature was 96° F. were 102.3° F. for control cows, 105.0° F. for cows on the lower rate of thyroprotein feeding and 105.1° F. for the higher level.

Pulse rates showed the effect of the thyroprotein addition, averaging 81 for the controls, 83 for lower rate of treatment and 95 for the higher rate, although the groups were balanced in the experimental period. Blood pressure readings showed no particular differences between controls and experimental animals.

Blood studies of the serum calcium, serum inorganic phosphorus and the hemoglobin showed no differences attributable to thyroprotein feeding. Blood sugar showed increases over the preliminary period averaging 21 and 19 per cent for the lower and higher levels of thyroprotein, compared to 7.0 per cent for the controls. The cows getting 2.00 g. thyroprotein per 100 lb. body weight showed the highest blood sugar levels and averaged 65.7 g. blood sugar per 100 ml. of whole blood, compared to 47.1 mg. for these same cows in the preliminary period.

#### SUMMARY

Thyroprotein was fed in midsummer to four cows on a 15-wk. double reversal study at a rate of 1.5 g. per 100 lb. of body weight, and at levels of 1.33 g. and 2.0 g. to six cows on 8-wk. continuous study, using two controls.

1. F.C.M. production increases noted were 32 per cent on reversal and 12 and 22 per cent on continuous as compared to preliminary period with an 8 per cent loss for controls on the latter study.

2. Efficiencies of T.D.N. utilization, using formula table 1 were 69 per cent for thyroprotein feeding periods compared to 104 per cent on basal ration for reversal trial; and 71 and 69 per cent respectively for continuous study, with 99 per cent efficiency for control pair.

3. Several cows receiving thyroprotein improved in efficiency of T.D.N. utilization as the period of thyroprotein feeding proceeded. Rapid body weight losses occurred in both trials when thyroprotein was fed, even when additional T.D.N. was provided. When thyroprotein was withdrawn from the ration of two cows on continuous trial they suffered heavy losses in milk yield and made unusually rapid and efficient weight gains over a 21-day period during cooler weather. These rapid gains and losses raise questions concerning the type of tissue involved.

4. Accelerated respiratory rates and pulse rates, as well as increased body temperatures, offered partial explanation for the reduced efficiency of energy utilization by the thyroprotein-fed cows during this summer trial.

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# MOTILITY OF SPERMATOOZOA AND CONTROL OF BACTERIA IN BOVINE SEMEN EXTENDERS CONTAINING SULFANILAMIDE, POLYMYXIN AND AUREOMYCIN

R. H. FOOTE AND R. W. BRATTON

*Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry,  
Cornell University, Ithaca, N. Y.*

The importance of controlling bacterial growth in bovine semen used for artificial insemination or for fundamental metabolic studies has been pointed out by Salisbury *et al.* (16). It has been reported that the growth of many organisms commonly found in bovine semen can be inhibited by the addition of sulfonamides (6, 8, 12, 15), of penicillin (3, 7, 10), of streptomycin (2, 9, 15) and of penicillin plus streptomycin (1) without harmful effects on the motility of the spermatozoa. However, no data have been reported on the control of bacterial growth in semen by the recently discovered polymyxins or aureomycin.

The polymyxins A, B, C, D and E are bactericidal against Gram-negative bacteria. Long *et al.* (14) state that they are "definitely more effective than is streptomycin against susceptible bacteria". Stansly *et al.* (18) have shown that such organisms as *Escherichia coli*, *Aerobacter aerogenes* and *Pseudomonas aeruginosa*, which frequently are found in the semen of bulls, readily yield strains resistant to streptomycin, but under the same conditions do not yield strains resistant to polymyxin. Another antibiotic, aureomycin, has been found to be bacteriostatic to many Gram-positive and Gram-negative bacteria (4, 14), and it is the first antibiotic known to affect several large viruses (14). Lacy and Lankford (13) have found it to be more effective than streptomycin against 26 cultures of *Brucella*. In view of the reported high bactericidal action of polymyxin and aureomycin against a number of pathogens commonly found in semen and associated with breeding troubles, it seemed desirable to establish drug concentrations which would not impair the motility of the spermatozoa and to study bacterial growth at these levels.

## MATERIALS AND METHODS

Three types of polymyxin, D, B and E, were used in these investigations. Polymyxin D has been described in detail by Stansly *et al.* (18) and B and E by Brownlee and Jones (5) and Jones (11). The hydrochloride form of aureomycin was used.

To make a simultaneous comparison of the effects of the antibiotics on the motility of spermatozoa and upon bacterial control, semen samples were divided into as many equal portions as there were levels of antibiotics chosen to be tested. Each portion of semen was mixed with 3.6 per cent citrate-yolk extender to which the antibiotics or sulfanilamide had been added. The average extension

rate was 1 part of semen to 100 parts of extender. The extended semen then was stored at 5° C. in 3-ml. test tubes filled to capacity. Daily microscopic examinations of the extended semen afforded an estimate of the effects of the antibacterial agents on the motility of the spermatozoa. Statistical significance between means was tested by analysis of variance (17).

The number of living bacteria was determined by the plate count method. Beef infusion agar containing 0.25 per cent yeast extract and 0.05 per cent glucose was used to culture the bacteria. Control plates were made to check the sterility of the water used for dilutions, of the agar and of the atmosphere in the laboratory during the plating procedure. All plates were incubated at 37° C. for 96 hr. before counting.

TABLE 1

*Motility of spermatozoa and numbers of bacteria in extended semen containing polymyxin D. (Av. of 7 ejaculates)*

Duration of storage at 5° C. (days)	Control SA 300 mg. %	μg.* polymyxin/ml. of extended semen				
		0	500	1000	2500	5000
Per cent of motile spermatozoa						
0	64	60	61	63	66	66
1	67	60	66	61	63	63
2	63	59	57	59	61	59
3	60	53	53	54	56	57
4	51	49	40	50	49	44
6	44	39	40	41	40	37
8	39	40	36	37	35	27
10	34	29	28	28	26	21
12	28	26	19	21	13	6
14	18	13	6	9	6	4
1,000's of bacteria/ml.						
14	4.2	6,700	6.9	4.6	3.0	1.2

\* The polymyxin sample was approximately 75% pure, so  $0.75 \times \mu\text{g.}$  used = equivalents of pure standard.

## RESULTS

*Polymyxin.* In the first experiment, 11 semen samples were stored at 5° C. in citrate-yolk containing 0, 5, 25, 125 and 625 μg. of polymyxin D. Citrate-yolk containing 300 mg. per cent of sulfanilamide was used as the control. Microscopic examinations for 5 consecutive days indicated that none of these levels of polymyxin decreased the motility of the spermatozoa. In the second experiment, levels of 0, 500, 1000, 2500 and 5000 μg. of polymyxin D per ml. of extender were studied. Their effect upon the motility of spermatozoa and the control of bacteria in seven semen samples is shown in table 1. In all cases polymyxin D was effective in controlling bacterial growth. No immediate toxic effects on the spermatozoa were noted, but during the latter part of the storage period the per cent of motile spermatozoa surviving in the presence of high concentrations of polymyxin was considerably less than in the control.

Other experiments in this laboratory comparing polymyxin D, B and E indicated that these three polymyxins were similar in their effects on spermatozoan motility and bacterial growth. When added to the citrate-yolk extenders at levels as high as 2000  $\mu\text{g.}$  per ml., all three were non-toxic to the spermatozoa, but were highly bactericidal. Brownlee and Jones (5) have reported that all polymyxins appear to have a similar bacterial spectrum.

*Aureomycin.* Aureomycin is unstable in alkaline solutions, but is stable for about 1 wk. at 5° C. in aqueous solutions of pH 2.5. Aureomycin added at the rate of 1000  $\mu\text{g.}$  per ml. of citrate-yolk extender (pH 6.8) and stored for 15 hr. at 5° C. was just as effective against the bacteria present in 11 semen samples as was a freshly prepared solution of aureomycin of the same strength. However, this level of aureomycin was toxic to the spermatozoa. Likewise, 500  $\mu\text{g.}$  of aureomycin per ml. of extender were found to be both bactericidal and spermicidal. In an effort to establish levels of aureomycin which would not be harmful

TABLE 2  
*The effect of aureomycin on motility of spermatozoa and bacterial growth*  
(Av. of 10 ejaculates)

Duration of storage at 5° C. (days)	Control SA 300 mg. %	μg. of aureomycin/ml. of extender			
		0	100	200	500
Per cent of motile spermatozoa					
0	71	69	69	68	68
1	66	69	67	67	64
2	61	62	61	57	52
4	54	52	50	39	31
1000's of bacteria per ml. after 24 hr. at 5° C.					
1	0.19	3.4	0.36	0.21	0.22
1000's of bacteria per ml. after 24 hr. at 20° C.					
1*	5.9	430.	7.3	6.5	6.3

\* At 20° C.

to the spermatozoa, aureomycin was added at the rate of 0, 100, 200 and 500  $\mu\text{g.}$  per ml. of the citrate-yolk extender. The citrate-yolk extender containing 300 mg. per cent of sulfanilamide was used as the control. Each of 20 semen samples was divided into five equal parts and each part added to one of the extenders being tested. The first ten samples were stored at 5° C. To provide an opportunity for greater bacterial growth, the next ten samples were stored at 20° C. Platings were made on all samples after 24 hr. of storage since most semen is used for insemination after approximately 24 hr. of storage. The per cent of motile spermatozoa was estimated microscopically in the samples stored at 5° C. The results are in table 2. After 1 day of storage the levels of 200 and 500  $\mu\text{g.}$  of aureomycin per ml. of extended semen were harmful to the spermatozoa ( $P < 0.05$ ). All levels of aureomycin tested and the sulfanilamide control effectively reduced bacterial growth at 5 and at 20° C., as compared to the

growth when no antibacterial agent was added. Therefore, aureomycin may be used to inhibit bacterial growth in extended semen, but to avoid spermicidal effects the dosage should not exceed 100  $\mu$ g. per ml. of extended semen.

#### SUMMARY

The effect of polymyxins D, B and E and aureomycin on the motility of bovine spermatozoa and upon the control of bacterial growth in bovine semen extended with citrate buffered yolk and stored at 5° C. was investigated. It was found that 2000  $\mu$ g. of polymyxins D, B or E, or 100  $\mu$ g. of aureomycin could be added per ml. of extender without spermicidal effects. These levels of antibiotics were highly bacteriostatic and/or bactericidal.

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# THE FERTILITY OF BOVINE SEMEN IN EXTENDERS CONTAINING SULFANILAMIDE, PENICILLIN, STREPTOMYCIN AND POLYMYXIN

R. H. FOOTE AND R. W. BRATTON

*Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry,  
Cornell University, Ithaca, N. Y.*

The effect of sulfanilamide, penicillin and streptomycin, singly and in combinations, on the fertility of bovine semen is controversial. Almquist (3) has reported no significant improvement in fertility when semen from high fertility bulls was treated with penicillin. On the other hand, Almquist (1, 2) has reported large increases in fertility accompanying the addition of penicillin, streptomycin, and penicillin plus streptomycin to semen from low fertility (problem) bulls, but no increase accompanying the use of sulfanilamide alone. Mixner (9) observed that when sulfanilamide or sulfanilamide plus penicillin was added to the extended semen of highly fertile bulls, the average fertility level was 7 percentage units higher than when penicillin alone was added. However, this difference was not significant statistically. Since the experiments to be reported herein were completed, Mixner (10) has reported that the addition of streptomycin or streptomycin plus penicillin to citrate-sulfanilamide-yolk extender did not increase the fertility of bovine semen. In contrast, Easterbrooks *et al.* (5) recently have reported a significant average increase in fertility accompanying the addition of streptomycin to the extended semen of bulls in general. The studies reported in this paper were conducted to obtain more information on the possible value of penicillin, streptomycin, polymyxin and sulfanilamide for improving the fertility of bulls with histories of relatively low fertility as well as bulls with histories of high fertility, when their semen was extended in citrate-yolk containing non-spermicidal quantities of these antibacterial agents.

## EXPERIMENTAL PROCEDURE

Two groups of nine bulls each were selected from the active stud of the New York Artificial Breeders' Cooperative, Inc. One group was comprised of bulls with histories of high fertility, while the other group was comprised of bulls of somewhat lower average fertility. On the basis of the 60- to 90-day non-returns to first service cows, the high fertility bulls averaged 64 per cent and the low fertility bulls 54 per cent during the 8 mo. preceding the experiment.

Sixty-four semen samples initially containing  $500 \times 10^6$  or more spermatozoa per ml. of which 50 per cent or more were estimated to be motile were used for insemination. All samples were partially extended immediately after collection at a rate of approximately 1:4 with warm (98° F.) 3.6 per cent citrate-yolk extender (7) and cooled to 5° C. in 75 min. After cooling they were divided into six portions and each portion extended to its final volume using the cold (5° C.) extenders composed of equal parts of fresh egg yolk and one of the six

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buffers shown in table 1. The levels of antibiotics used were based on the reports by Foote (6) and Foote and Bratton (8). Final extension rates were adjusted to give approximately  $15 \times 10^6$  motile spermatozoa per ml. of extended semen. All extenders were prepared during the afternoon of the day previous to their use and were stored at 5° C. in the dark until used.

Inseminations were made by the regularly employed technicians associated with the New York Artificial Breeders' Cooperative, Inc. Semen from each bull was shipped a sufficient number of times to allow all technicians equal opportunities for using each bull and each extender.

Fertility was measured by the per cent of first and second service cows not returning to artificial service within 60- to 90-days after the month in which they were bred. The per cent non-returns calculated for each semen sample  $\times$  treatment (experimental extender) sub-class was used as the experimental unit in the analysis of variance (13).

TABLE 1  
*Composition of experimental buffers*

Ingredients	Buffers					
	1	2	3	4	5	6
Sodium citrate dihydrate, g.	3.6	3.6	3.6	3.6	3.6	3.6
Sulfanilamide, g.		0.6				0.6
Penicillin G sodium crystalline (Merck), Oxford units			100,000			100,000
Streptomycin, calcium chloride complex (Merck), $\mu$ g.				100,000		100,000
Polymyxin sulfate, $\mu$ g.					100,000	100,000
H <sub>2</sub> O, distilled over glass to vol., ml.	100	100	100	100	100	100

#### RESULTS AND DISCUSSION

The number of first and second service cows inseminated and the mean percentages for the 60- to 90-day non-returns for these cows are shown in table 2. The analysis of variance of the per cent non-returns to first service cows revealed a significant treatment  $\times$  fertility group interaction ( $P < 0.05$ ). The treatment means for the per cent non-returns were not significantly different for the high fertility group of bulls. On the other hand, the addition of penicillin, streptomycin or the combination of all antibacterial agents to the extended semen of the low fertility group of bulls was accompanied by non-return rates that averaged approximately 10 percentage units higher than those accompanying the use of sulfanilamide, polymyxin or no antibacterial agent. On the basis of the combined first and second service cows for both high and low fertility groups of bulls the  $F$  value for the differences between the means for extenders was slightly less than that required for significance at the 5 per cent level of probability.

In this experiment there was only a small difference between the non-return percentages for the 3.6 per cent citrate-yolk and 3.6 per cent citrate-sulfanilamide-

yolk. This difference is in contrast to results previously reported from this laboratory (4, 11, 12). However, it is in agreement with more recent unpublished findings in this laboratory (14), which suggest that the cooling procedure (7) accompanying the processing of semen may accomplish an increase in fertility similar to that attributed to sulfanilamide (4, 12).

The results obtained in these studies, together with those obtained by Almquist (1, 2) and Easterbrooks *et al.* (5), appear to warrant the use of penicillin, streptomycin or a combination of these plus polymyxin and sulfanilamide in extenders for bovine semen for the purpose of increasing, to a limited extent, the over-all fertility level of bovine semen used for artificial breeding.

TABLE 2

*Average fertility of extended semen containing sulfanilamide, penicillin, streptomycin, polymyxin and combinations of these antibacterial agents based on 60- to 90-day per cent non-returns to first and second service cows*

Cow group	Bull fertility group		Extenders					
			No anti-biotic	Sulfanilamide	Penicillin	Streptomycin	Polymyxin	All combined
1st service cows	High	No. serv.	334	341	336	352	356	326
		% N.R.*	65	66	71	69	67	68
	Low	No. serv.	334	309	281	292	245	277
		% N.R.	58	61	68	69	61	73
	Combined	No. serv.	668	650	617	644	601	603
		% N.R.	62	64	69	69	64	71
1st and 2nd service cows	High	No. serv.	472	506	472	488	499	452
		% N.R.	63	64	70	69	68	67
	Low	No. serv.	461	421	413	419	363	393
		% N.R.	58	60	67	66	59	69
	Combined	No. serv.	933	927	885	907	862	845
		% N.R.	61	62	68	66	64	68

\* N.R. = non-returns.

## SUMMARY

By use of the split sample technique the fertility of bovine semen extended in 3.6 per cent citrate-yolk containing no antibacterial agent, sulfanilamide, penicillin, streptomycin, polymyxin or a combination of these four antibacterial agents was studied. Semen was used from bulls with histories of low fertility, as well as from bulls with histories of high fertility.

The per cent 60- to 90-day non-returns to first and second service cows for the treatments, no antibacterial agent, sulfanilamide, penicillin, streptomycin, polymyxin, and sulfanilamide plus penicillin plus streptomycin plus polymyxin were, respectively, for the high fertility bulls, 63, 64, 70, 69, 68, and 67; for the low fertility bulls, 58, 60, 67, 66, 59, and 69 and for both the high fertility and the low fertility bulls combined, 61, 62, 68, 68, 64, and 68. On the bases of these results and those reported by other workers, it is concluded that the addition of penicillin, streptomycin or a combination of these plus polymyxin and sulfanila-

mide to present day extenders may be expected to increase the over-all fertility level of bovine semen used for artificial breeding.

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# CARBOHYDRATE UTILIZATION IN THE YOUNG CALF. I. NUTRITIVE VALUE OF GLUCOSE, CORN SYRUP AND LACTOSE AS CARBOHYDRATE SOURCES IN SYNTHETIC MILK<sup>1</sup>

R. J. FLIPSE<sup>2,3</sup>, C. F. HUFFMAN, H. D. WEBSTER  
AND C. W. DUNCAN

*Departments of Dairy, Animal Pathology, Agricultural Chemistry and the Institute of Nutrition,  
Michigan Agricultural Experiment Station, East Lansing*

The problem of a satisfactory milk replacement for the raising of calves is a matter of utmost economic importance to the dairyman. Many calf starters have been developed but, in general, success appears to depend upon the use of at least 300 lb. of whole milk and the inclusion of dried milk products in the starter (7, 11, 13, 24, 25). Synthetic milks in themselves are far from being the answer to the economic problem in that the cost of purified components is much greater than the cost of whole milk. Yet fundamental problems can be approached through this means which can not be studied with calves on natural feeds. Early attempts to raise calves on purified diets were unsuccessful (8) but Wiese *et al.* (23) have reported the formulation of a synthetic diet satisfactory for the nutrition of the young calf.

In this investigation an attempt was made to determine the relative efficiency of certain carbohydrates incorporated in synthetic milks for calves. Since Wiese *et al.* (23) were successful with glucose, this sugar was selected for use as the control. Lactose, the natural carbohydrate source of the neonatal mammal, and Karo corn syrup, frequently used in formulas for infants, were selected for comparison with glucose.

## EXPERIMENTAL PROCEDURE

*Selection of animals.* The composition of the three experimental groups is indicated in table 1. All of the 18 experimental calves were males, with the exception of one female in group G. The system used in assigning calves to the groups and subgroups consisted of random allotment as the calves were born in the College experimental herd. The only prerequisite to assignment was normal health and appearance. Calves were placed on experiment 2 to 3 days after birth and retained for a 31-day feeding trial, since the first month is the critical period with respect to carbohydrate utilization in the calf (19). Following the feeding trial, autopsy was performed on most of the animals, al-

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<sup>3</sup> Present address: Department of Dairy Husbandry, The Pennsylvania State College, State College.

though a few were returned to the College experimental herd for subsequent research.

*Feeding and management.* Of necessity, calves were started on the experiment in all seasons of the year. Possible differences due to prenatal nutrition were minimized, since the dams were stall-fed throughout the year. Calves were permitted to remain with their dams for 12 hr. following parturition. Subsequently, each calf was placed in an individual pen, starved 14 to 24 hr.

TABLE 1  
*Composition of the experimental groups*

Group	Sub-group	No. of calves	Breed Distribution	Average starting wt.
				(lb.)
G (glucose)		6	5 Holstein, 1 Ayrshire	94.3
	10	2	1 Jersey, 1 Brown Swiss	82.0
K (corn syrup)	30	2	2 Holstein	96.5
	45	2	1 Jersey, 1 Brown Swiss	75.5
	5	2	1 Holstein, 1 Jersey	67.0
L (lactose)	10	2	2 Holstein	81.5
	30	2	1 Holstein, 1 Jersey	69.5

and started on the synthetic milk diet. Feed was given twice daily via nipple pail at a rate calculated to meet the recommended nutrient allowances of the National Research Council (9).

The constituents of each of the rations fed are listed in table 2, and the chemical analyses of these rations are presented in table 3. In addition to the

TABLE 2  
*Ingredients of the rations fed*

Group	Ration						
	G	K				L	
Subgroup		10	30	45	5	10	30
Glucose	60	50	30	15	55	50	30
Corn syrup		10	30	45			
Lactose					5	10	30
Casein	25	25	25	25	25	25	25
Lard	10	10	10	10	10	10	10
Salts <sup>a</sup>	5	5	5	5	5	5	5

<sup>a</sup> The salt mixture was composed of 10 parts  $\text{CaCO}_3$ , 20 parts  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ , 20 parts  $\text{Mg}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ , 10 parts  $\text{K}_2\text{HPO}_4$ , 5 parts  $\text{NaCl}$ , 5 parts  $\text{KCl}$ , 1.98 parts  $\text{FeC}_6\text{H}_5\text{O}_7 \cdot \text{XH}_2\text{O}$ , 0.04 parts  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.04 parts  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 0.04 parts  $\text{CoSO}_4 \cdot 4\text{H}_2\text{O}$ .

components listed, each calf received (a) a capsule containing 70,000 I.U. of vitamin A (shark liver oil) and 10,000 I.U. of vitamin D (viosterol) at the time it was placed on experiment and at weekly intervals thereafter and (b) a daily dosage of 20 mg. thiamin hydrochloride, 20 mg. riboflavin, 20 mg. calcium pantothenate, 20 mg. nicotinic acid, 20 mg. para-aminobenzoic acid, 5 mg. pyridoxine hydrochloride, 10 mg. vitamin K, 1 mg. biotin, 200 mg. inositol and 3 g. choline chloride. These water soluble vitamins were prepared in stock solu-

tion, stored in amber glass under refrigeration and added to the synthetic milk at the time of the morning feeding.

The carbohydrate content of the rations was varied in the different groups. In the *G* group, which was used as the control, glucose was the carbohydrate source; various amounts of glucose were replaced with corn syrup in the *K* group and various amounts of glucose were replaced with lactose in the *L* group, as shown in table 2.

Calf pens were bedded with wood shavings. No hay was fed, and in order to minimize consumption of shavings, each calf received daily 1 oz. of a mixture containing 10 per cent cellulose, 57 per cent glucose, 24 per cent casein, 5 per cent salts and 4 per cent diluted corn syrup. With few exceptions calves ate this dry mix readily and showed slight inclination to consume shavings.

*Preparation of feed.* The synthetic milk was prepared by a modification of

TABLE 3  
*Chemical analysis of the rations fed*

Group	Ration						
	G		K		L		
	Subgroup		10	30	45	5	10
Moisture (%)	7.22	8.51	11.02	13.06	6.95	6.68	5.62
Protein (%)	21.39	21.36	21.30	21.26	21.38	21.38	21.37
Ash (%)	5.64	5.73	5.92	6.06	5.64	5.64	5.65
Crude fiber (%)	0.11	0.10	0.08	0.06	0.11	0.10	0.08
Ether ext. (%)	10.34	10.31	10.21	10.15	10.34	10.34	10.34
N. F. E. (%)	55.30	53.99	51.47	49.41	55.58	55.86	56.94
Ca (%)	0.65	0.65	0.66	0.67	0.65	0.65	0.65
P (%)	0.77	0.77	0.78	0.78	0.77	0.77	0.77
Mg (%)	0.31	0.32	0.35	0.37	0.31	0.32	0.32
K (%)	0.52	0.55	0.61	0.65	0.52	0.52	0.52
Fe (%)	0.0014	0.0016	0.0020	0.0023	0.0014	0.0014	0.0014
Cu (ppm.)	2.1	2.1	3.9	4.8	2.2	2.2	2.4
Mn (ppm.)	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Co (ppm.)	0.115	0.117	0.121	0.124	0.116	0.118	0.124

the procedure of Wiese *et al.* (23). Due to the limited refrigeration facilities available at the experimental barn, the synthetic milk was prepared once or twice weekly and stored as a liquid concentrate. The frequency of preparation depended upon the number of calves on trial at any particular time. The liquid concentrate was prepared as follows: Four ounces of sodium bicarbonate were dissolved in 43 lb. of water at 60° C. A heavy-duty electric stirrer was used and 5 lb. of casein were added slowly with constant agitation. Stirring was continued 20 to 30 min. to insure complete solution of the casein. Near the end of the agitation period 2 lb. of lard (80° C.) were thoroughly mixed with the casein solution. The casein-lard solution was homogenized at 3,000 lb. pressure. The remaining components of the ration (carbohydrate(s) and salts, table 2) were mixed dry and 12.5 lb. of this dry mix were blended with the 50 lb. of the homogenized solution. The synthetic milk liquid concentrate thus prepared contained 33.3 per cent dry matter and was stored under re-

frigeration in this form. At feeding time one part of the concentrate was added to two parts of hot water, the vitamin solution added (mornings only) and the product fed at 85 to 95° F.

*Criteria for evaluation of response.* Evaluation of the response to the experimental rations was based upon (a) observations on the health and general appearance, (b) growth and efficiency of feed utilization, (c) blood analyses, (d) rumen population and (e) post-mortem examinations.

(a) *Health and general appearance.* Observations and recordings were made at least once daily with regard to general condition, appetite and general reactions of the animals and the consistency of the feces.

(b) *Growth and efficiency of feed utilization.* An accurate tabulation of feed consumption and refusal was maintained. Each calf was weighed prior to the morning feeding on the day it was placed on the experiment and on the 4th, 7th, 11th, 14th, 18th, 21st, 25th, 28th and 31st days of the trial.

(c) *Blood analyses.* Blood samples were collected from the jugular vein of each calf at weekly intervals. Determinations of hemoglobin and hematocrit were made on whole blood by the methods of Sanford *et al.* (18) and Wintrobe (26), respectively. The plasma was analyzed for calcium, inorganic phosphorus, magnesium (5) and ascorbic acid (10).

(d) *Rumen population.* Samples of the rumen contents were obtained from most of the calves at weekly intervals. A few calves objected to the passage of the stomach tube and no attempt was made to force collection from such calves. The collections were made 4 hr. after the morning feeding. The samples were preserved in an aqueous solution of formaldehyde and counts were made of iodophilic organisms (1) and total bacteria (21) per milliliter of rumen contents.

(e) *Post-mortem examinations.* Animals which died during the trial or were killed at the end of the experimental period were subjected to gross postmortem examinations. Histological sections were made of selected organs from representative animals and of any organ or tissue which appeared abnormal in the gross inspection.

## RESULTS

*Health and general appearance.* Within 2 to 4 days after being placed on the experiment, feces from calves of the *G* (glucose) and *K* (corn syrup) groups invariably would become quite soft and in many cases semiliquid. The *K* group was much more severely afflicted than was the *G* group. On the other hand, *L* (lactose) calves, even at the 5 per cent level, maintained normal consistency of feces throughout the trial. The *L* calves, in general, possessed smoother hair coats and showed more alertness than animals in the other groups. Calves on corn syrup, though their weight gains compared favorably with those of calves on glucose, characteristically had much duller hair coats than the latter. All calves drank the synthetic milk readily. Bloat occurred in two glucose calves, and one calf receiving the 10 per cent level of lactose died of acute bloat of the abomasum.



**Growth and feed utilization.** The gain in body weight and the efficiency of feed utilization of each of the experimental groups and subgroups is indicated in table 4. In view of the extensive variation in the starting weight, gains are represented both as pounds and as percentage increase over the starting weight.

**Blood analysis.** The average values for hematocrit, hemoglobin and plasma calcium, inorganic phosphorus, magnesium and ascorbic acid for each group are presented graphically in figure 1. These values appear to be within the normal range for calves of this age (2, 28). There is little apparent difference between groups in any of the constituents, although the plasma ascorbic acid tends to be slightly lower in the corn syrup-fed calves than in the other groups.

**Rumen population.** A total of 38 rumen samples were collected from the three groups. The average iodophil counts (millions per ml.) were 423.0, 463.1 and 215.9, while the average total counts (millions per ml.) were 2,800, 4,222 and 2,400 for the *G*, *K* and *L* groups, respectively. However, the variations

TABLE 4  
*Growth and feed utilization*

Group	Sub-group	Average gain		Gain/lb. DM consumed
		(lb.)	(%)	(lb.)
G	Av.	9.33 ± 1.58 <sup>a</sup>	8.13	0.234 ± 0.055
K	10	15.50	18.90	0.240
	30	14.50	15.03	0.231
	45	-4.00	-5.30	-0.044
	Av.	8.66 ± 5.68	10.23	0.142 ± 0.092
L	5	16.00	23.88	0.306
	10	20.50	25.15	0.508
	30	19.50	28.06	0.338
	Av.	18.66 ± 2.73	25.92	0.384 ± 0.050

<sup>a</sup> Standard error of the mean.

obtained between calves within groups and between samples from the same calf on different dates were so great that no significance can be attached to the means.

**Post-mortem examination.** Ten of the 18 experimental calves were subjected to post-mortem examination, either during the experimental period or at the completion of the trial, with the exception of one calf which was examined 10 days after completion of the trial. Two lactose calves and four from each of the remaining groups were autopsied during this period. Congestion and consolidation of lung tissue was the most common finding, although focal interstitial nephritis (white spotted kidney) also was prevalent. Focal interstitial nephritis occurred irrespective of the dietary regime. Congestion and consolidation of lung tissue occurred with equal frequency in the glucose and corn syrup groups, but during the experimental period only one of the lactose calves had a "cold," which was successfully treated with sulfathiazole. Ulceration of the pylorus, petechial hemorrhages of the abomasum and patchy congestion of the intestinal tract occurred frequently in the corn syrup and glucose groups,

but less frequently and less severely in the lactose group. The previously mentioned lethal bloat in one lactose calf resulted in rupture of the abomasal wall.

#### DISCUSSION

Although growth responses were satisfactory in at least one of the groups, the reaction of calves to the synthetic milk hardly can be considered normal. The high incidence of respiratory disturbances, as evidenced by visible symp-

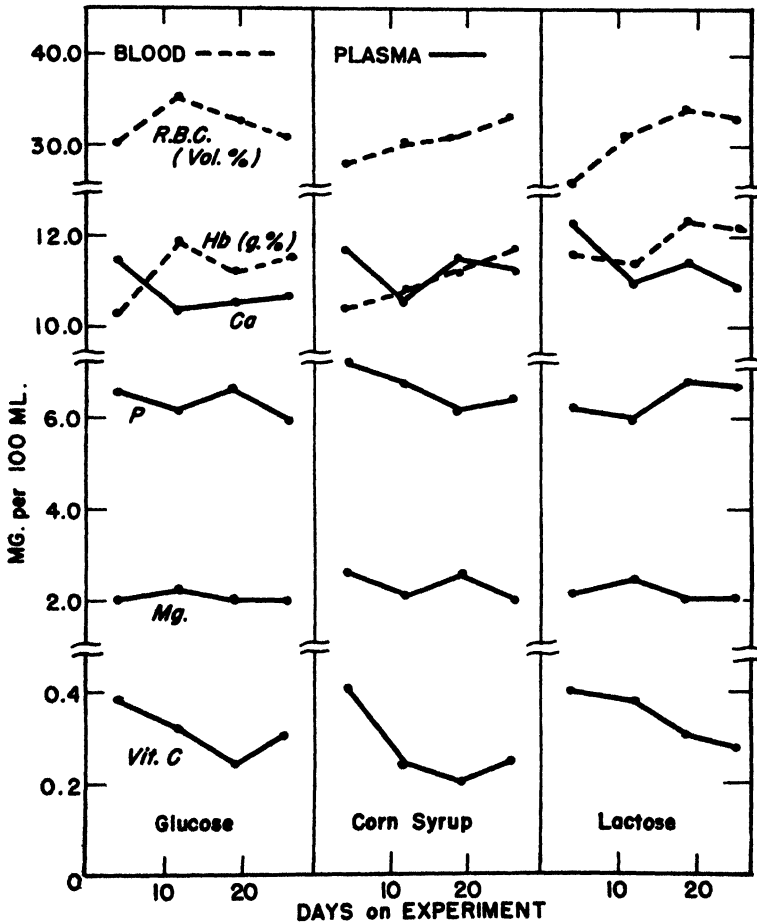


FIG. 1. Changes in the composition of the blood of calves.

toms as well as post-mortem findings, indicates that the ability of the calves to resist infection was low; however, the physical condition of the calves in the lactose group was superior to the calves in the other groups. The incidence of focal interstitial nephritis also was high, but the etiology of this condition is vague. Moore and Hallman (12) found it associated with low vitamin A in-

take in young calves, while Smith (20) reported the deprivation of colostrum as a predisposing factor.

The blood data indicate that sufficient calcium, phosphorus and magnesium were absorbed to maintain the levels of these elements in the plasma. The reported rise in plasma inorganic phosphorus during the first 3 wk. of life (15, 28) was not apparent in these experimental animals. Likewise, the normal decline in hemoglobin concentration and in hematocrit (28) was not evident in these trials. Such a decline possibly was prevented by the inclusion of iron and trace elements in the synthetic milk, although Davidson and Leitch (4) report other than the reduction in hemoglobin and hematocrit following birth is independent of iron reserves and of dietary intake. This does not preclude the possibility that one or more of the trace elements included in the ration may have prevented the decline.

Corn syrup, a food substance widely used in formulas for infants, was selected as a promising carbohydrate for the neonatal calf. The results refuted pre-experimental expectations. Although growth at the 10 and 30 per cent levels compared favorably with other groups, the feces were of foul odor and of semiliquid consistency. Apparently, the laxation produced by corn syrup offset any beneficial nutritive value it may have possessed. Chemical analyses (table 3) indicated that corn syrup was high in ash. It is possible that this ash contained some element(s) which caused the laxation and thus prevented the animals from utilizing properly the feed consumed.

The beneficial effect of lactose on the intestinal tract, as evidenced by the consistency of feces, was striking, and results of this benefit are reflected in the growth response and in the efficiency of feed utilization. Much of the beneficial action of lactose may be credited to its stability and low solubility which permits it to pass unchanged into the intestine. In the intestine it promotes the growth of beneficial lactic acid-producing bacteria and inhibits scatologic putrefaction (3, 14). Although this view is not universally accepted (16, 22), it has much in its favor and must be considered until a more satisfactory explanation is advanced.

Rumen bacterial samples were collected for study as a possible index to the time at which the rumen commences to function, but these samples revealed little difference among the groups. However, the intestinal flora are probably of much greater importance in controlling gastro-intestinal motility than are those of the rumen. A recent review (6) adequately discusses the effect of lactose on gastro-intestinal motility and indicates that there is more evidence supporting the contention that lactose tends to cause diarrhea than there is evidence to the contrary. Within the bovine species, Wise (27) has used dried whey in treating chronic diarrhea in calves and Rojas *et al.* (17) have indicated that under normal feeding conditions lactose was utilized effectively by calves. It was only when the lactose content of milk was doubled that diarrhea and unthriftiness occurred and the efficiency of utilization was decreased markedly. The results of feeding lactose in synthetic milk are in harmony with the report of Rojas *et al.*, since in all cases the lactose content of synthetic milk was below the level contained in normal cow's milk.

Whatever the mode of action of lactose, the quantity required by the calf is small, because 5 per cent produced almost as satisfactory growth response as did 30 per cent. Although the difference was small, the greatest efficiency of feed utilization was obtained at the 10 per cent level of lactose. Whether the decrease in efficiency at the 30 per cent as compared to the 10 per cent level is due to too high a proportion of lactose or to biological variation is open to speculation.

#### SUMMARY

Eighteen neonatal calves were allotted to three experimental groups and fed rations consisting of synthetic milks which varied only in the source of carbohydrate.

The average gain in weight for the 31-day experimental period was 9.33 lb. for glucose-fed calves (*G*), 8.66 lb. for corn syrup-fed calves (*K*) and 18.66 lb. for lactose-fed calves (*L*). The efficiency of feed utilization, expressed as the average of gain per pound of dry matter consumed, was 0.234, 0.142 and 0.384 for the *G*, *K* and *L* groups, respectively. Within the subgroups, 10 and 30 per cent corn syrup produced fair results, while 45 per cent was unsatisfactory. There was little difference in weight gains in response to lactose at the 5, 10 and 30 per cent levels.

Analysis of blood at weekly intervals for hematocrit, hemoglobin and plasma calcium, inorganic phosphorus, magnesium and ascorbic acid showed no apparent departure from the normal.

Examination of rumen samples for microorganisms revealed that individual differences were greater than differences between groups.

On post-mortem examination, pneumonia, patchy congestion of the intestinal tract, abomasal petechial hemorrhages and ulceration about the pylorus were observed less frequently in the *L* than in *G* and *K* groups, while focal interstitial nephritis occurred indiscriminately in all groups.

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# CARBOHYDRATE UTILIZATION IN THE YOUNG CALF. II. THE NUTRITIVE VALUE OF STARCH AND THE EFFECT OF LACTOSE ON THE NUTRITIVE VALUES OF STARCH AND CORN SYRUP IN SYNTHETIC MILK<sup>1</sup>

R. J. FLIPSE<sup>2, 3</sup>, C. F. HUFFMAN, C. W. DUNCAN AND H. D. WEBSTER

*Departments of Dairy, Agricultural Chemistry, Animal Pathology and the Institute of Nutrition, Michigan Agricultural Experiment Station, East Lansing*

Lactose has been shown to benefit the calf when it replaced glucose in synthetic rations in amounts as small as 5 per cent of the ration (5). Under practical feeding conditions the bulk of the carbohydrate in a calf starter consists of starch. The report of Shaw *et al.* (15) indicates that the calf is unable to digest starch efficiently until 4 to 5 wk. of age.

The investigation reported herein was undertaken to determine the effect of lactose on the utilization of such carbohydrate sources as starch and corn syrup in synthetic rations by the very young calf.

## EXPERIMENTAL PROCEDURE

Neonatal calves were randomized to the three experimental groups listed in table 1. As in the previous investigation (5), calves were started on the ex-

TABLE 1  
*Composition of the experimental groups*

Group	No. of calves	Breed distribution	Av. starting wt. (lb.)
KL (Corn syrup + lactose)	3	2 Holstein 1 Jersey	69.0
SL (Starch + lactose)	3	1 Holstein 1 Ayrshire 1 Jersey	77.0
S (Starch)	3	3 Holstein	95.0

periment as they were born in the College experimental herd. Details regarding the selection, feeding and management of animals and the preparation of feed have been described previously (5). The present study was initiated near the

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<sup>3</sup> Present address: Department of Dairy Husbandry, The Pennsylvania State College, State College.

close of the aforementioned investigation and, in regard to methods, was merely a continuation of it. Various amounts of carbohydrates were incorporated in the rations, as shown in table 2. The *KL* group received glucose, corn syrup and lactose as the carbohydrate sources, the *SL* group received glucose, lactose

TABLE 2  
*Ingredients of the rations fed*

Group	Ration		
	KL	SL	S
Glucose	5	5	15
Corn syrup	45		
Lactose	10	10	
Starch		45	45
Casein	25	25	25
Lard	10	10	10
Salts <sup>a</sup>	5	5	5

<sup>a</sup> The salt mixture was composed of 10 parts  $\text{CaCO}_3$ , 20 parts  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ , 20 parts  $\text{Mg}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$ , 10 parts  $\text{K}_2\text{HPO}_4$ , 5 parts  $\text{NaCl}$ , 5 parts  $\text{KCl}$ , 1.98 parts  $\text{FeO}_6\text{H}_5\text{O}_7 \cdot \text{XH}_2\text{O}$ , 0.04 parts  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.04 parts  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 0.04 parts  $\text{CoSO}_4 \cdot 4\text{H}_2\text{O}$ .

and starch, and the *S* group received glucose and starch. Composition and the chemical analyses of the rations fed are presented in tables 2 and 3, respectively.

Criteria for the evaluation of response were altered from those of the previous investigation in that no samples of rumen contents were collected and only one post-mortem examination was conducted. The single autopsy was performed on a member of the *S* group to check for lesions previously produced on a starch-containing synthetic milk (4).

TABLE 3  
*Chemical analysis of the rations fed*

Group	Ration		
	KL	SL	S
Moisture (%)	12.52	7.36	7.90
Protein (%)	21.25	21.57	21.57
Ash (%)	6.06	5.63	5.62
Crude fiber (%)	0.05	0.07	0.08
Ether ext. (%)	10.15	10.61	10.61
N. F. E. (%)	49.97	54.76	54.22
Ca (%)	0.67	0.65	0.65
P (%)	0.78	0.78	0.78
Mg (%)	0.38	0.34	0.33
K (%)	0.65	0.52	0.52
Fe (%)	0.0023	0.0022	0.0021
Cu (ppm.)	4.9	2.2	2.1
Mn (ppm.)	0.37	0.07	0.07
Co (ppm.)	0.127	0.118	0.115

In search of a possible explanation for varying responses to the carbohydrates fed, a test meal containing a single carbohydrate source (*e.g.*, glucose, lactose, starch or corn syrup) was fed to an animal, and the blood sugar concentration determined at intervals over an 8-hr postprandial period. The

test was repeated until a curve for each carbohydrate was obtained on three different calves. The test meal consisted of 25 per cent casein, 10 per cent lard, 5 per cent salts and 60 per cent of the carbohydrate being tested. Carbohydrates were calculated on the dry-matter basis to eliminate differences in carbohydrate intake caused by variable moisture content. This meal was prepared in the same manner as the usual experimental ration and fed according to the recommended allowances of the National Research Council (7). Interpolations of these recommended allowances were made to adjust feed intake to requirements based on the exact body weights of the calves. On the day of the test, blood was collected prior to the morning feeding. The test meal was fed and subsequent blood samples were taken at 0.25, 0.5, 1, 2, 4, 6 and 8 hr. postprandially. All blood samples were collected by jugular venipuncture with minimum disturbance to the animal. To facilitate rapid, efficient collection of blood, the neck was clipped closely a day or two prior to collection.

Potassium oxalate was used as the anticoagulant. Filtrates were prepared within 30 min. after the collection of blood and the amount of blood sugar was determined by the Somogyi procedure (16). Transmission was determined by a Cenco-Sheard spectrophotometer at 520  $m\mu$ .

TABLE 4  
*Growth and feed utilization*

Group	Average gain		Gain/lb. DM consumed
	(lb.)	(%)	(lb.)
KL	28.33 $\pm$ 5.85 <sup>a</sup>	41.06	0.487 $\pm$ 0.058
SL	24.67 $\pm$ 2.94	32.03	0.412 $\pm$ 0.072
S	14.00 $\pm$ 3.06	14.74	0.204 $\pm$ 0.046

<sup>a</sup> Standard error of the mean.

## RESULTS

*Health and general appearance.* The feces of calves in group *S* became very soft in consistency within 2 to 4 days after the animals were placed on experiment. The amount of feces voided was quite large, and the feces appeared to contain considerable undigested material. Calves of the *SL* group also voided excessive quantities of feces of semiliquid consistency, although, in general, the diarrhea in this group was not so severe as that in the *S* group. Diarrhea occurred infrequently in the *KL* group, in marked contrast to group *K* of the previous trial (5). Calves of the *S* group showed moderate emaciation and dehydration during the first 2 wk. of the trial, whereas those of groups *SL* and *KL* were relatively thrifty throughout the trial.

*Growth and feed utilization.* Gains in body weight for the 31-day period and the efficiency of feed utilization for each group are indicated in table 4. Calves of groups *KL* and *SL* gained in weight uniformly throughout the trial, whereas those in group *S* gained little or none during the first 2 wk., but they did increase in weight rapidly late in the experimental period.

*Blood analysis.* Average weekly levels of hemoglobin and cell volume



(hematocrit) and each of four plasma constituents are indicated in figure 1. These average values agree reasonably well with the normal levels for calves of this age (2, 18), although the hemoglobin levels tended to be higher and the inorganic phosphorus levels somewhat lower. Variation in serum magnesium (3) was greater than might be expected, but there did not appear to be any rela-

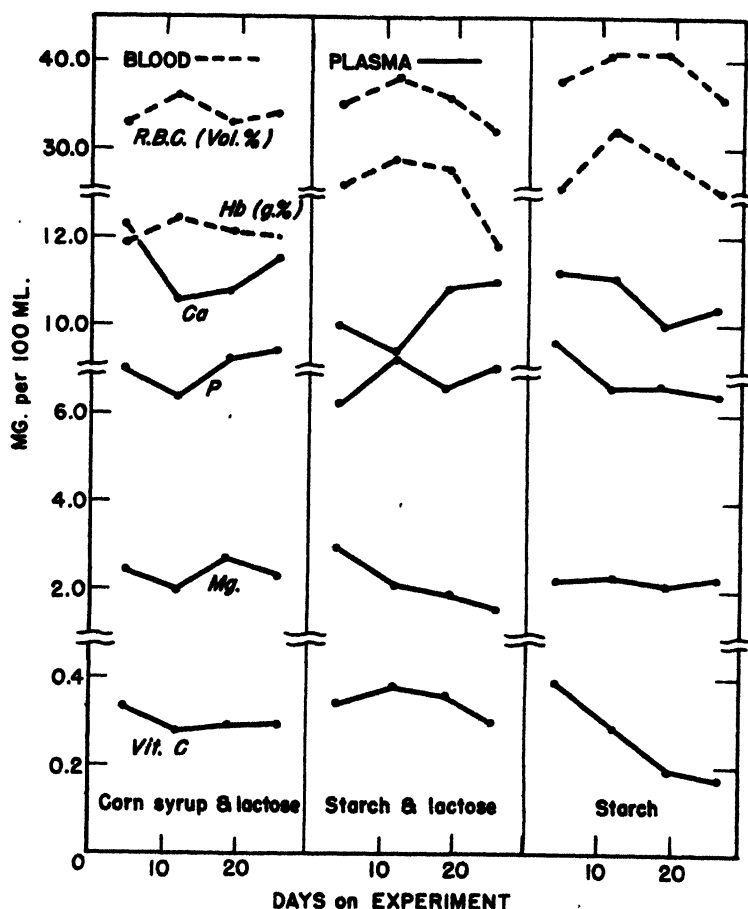


FIG. 1. Changes in the composition of the blood of calves.

tionship between the type of ration fed and the values obtained from the blood analyses.

Glucose absorption curves for each of the four carbohydrates tested, plotted from serial blood sugar determinations, are shown in figure 2. Blood sugar remained quite constant after starch ingestion and registered no increase during the first 4 hr. postprandially. Curves for the other three carbohydrates rose rapidly following feeding with little difference in evidence 30 min. after feed-

ing. Thereafter, the differences were striking. Corn syrup ingestion resulted in maximum blood sugar concentration 1 hr. after feeding, whereas the glucose or lactose peak was not reached until 4 hr. after feeding. The curve for glucose, however, descended more rapidly than did that for lactose. The maximum blood sugar concentration after corn syrup ingestion was only slightly more than half the maximum concentration following the consumption of glucose or lactose.

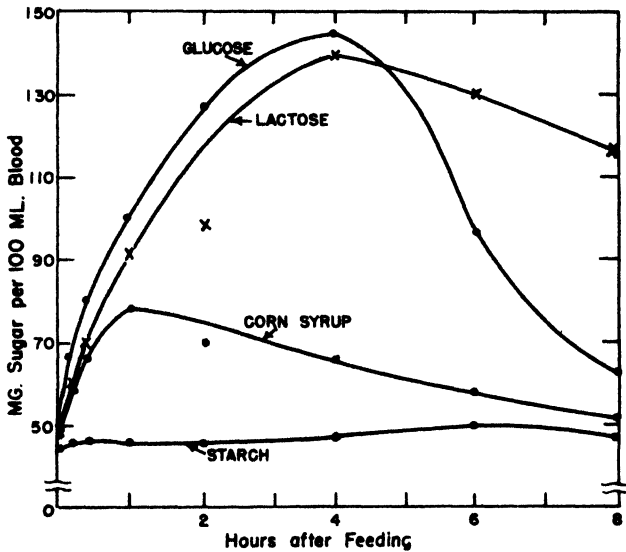


FIG. 2. Blood sugar concentration curves following ingestion of a single source of carbohydrate. Each curve is the average of three tests.

#### DISCUSSION

The single post-mortem examination performed was on a member of the S group and failed to reveal many of the lesions previously reported as characteristic of calves on a high-starch synthetic milk (4). Assuming that the animal examined was representative of the group, a possible explanation for the discrepancy in results may be found in the fact that in the earlier study the artificial milk contained natural feedstuffs high in starch. Pure corn starch added to these natural sources produced a higher level of starch feeding than was used in the present trial and may account for the differences noted in examining the experimental subjects.

The blood sugar level following starch ingestion showed no tendency to rise for the first 4 hr. after feeding (fig. 2). This might be anticipated, since starch is hydrolyzed slowly in the digestive tract. In fact, Shaw *et al.* (15) reported that the calf was unable to utilize starch to an appreciable extent until nearly 1 mo. of age. The glucose absorption curves obtained on a 2-wk.-old calf (4) substantiate this report in that there was no increase in blood sugar

over the 8-hr. sampling period. Since saliva of the bovine is devoid of amylolytic enzymes and pancreatic amylases are low in this species (17), the question is raised as to whether the failure of the neonatal calf to digest starch is due to (a) the inherent weakness of pancreatic amylase, with the result that the digestion of starch must await the development of starch-splitting microorganisms in the rumen, or (b) amylolytic activity of pancreatic (and/or possibly intestinal) juice is not developed at birth and fails to become efficient until several weeks after birth.

All data used in the preparation of figure 2 are from calves 28 to 35 days of age. The rapid rise in blood sugar following lactose ingestion is difficult to explain in that lactose presumably must be hydrolyzed before appreciable absorption can take place (12, p. 103). The continued high level of blood sugar would seem to indicate that the hydrolysis of lactose continues over a period of time. This may be considered to be advantageous in that energy is made available over an extended period and thus absorption and utilization are permitted to take place more efficiently. Blood sugar levels can be considered only as a rough index of the utilization of a carbohydrate. The concentration of blood sugar at any specified time depends upon the rate of absorption of the sugar into the blood stream and the rate at which it is removed from the blood. Removal may be accomplished through the kidneys or through utilization by the tissues, and the rate of removal, whether by excretion or utilization, varies with the sugar concerned (12, pp. 107, 133).

The alteration of response produced by adding lactose to either a corn syrup or a starch ration is remarkable. With corn syrup, the addition of lactose changed the response from no gain (5) to an average of over 28 lb. gain in 31 days (table 4). In the case of starch, lactose addition was accompanied by an increase in the rate of gain of 76 per cent (table 4). The differences are even more striking when one considers that the *KL* and *SL* groups surpassed not only the *K* and *S* groups in performance, but also the *L* group (5). Such results would not appear unusual in the study of proteins, in which the biological value of one protein may be increased greatly by the addition of a second protein (1). However, carbohydrates supposedly are hydrolyzed to the constituent monosaccharides before absorption can take place (12, p. 103) and supplementary relationships generally are not recognized in this class of compounds.

Although lactose possesses a lower specific dynamic effect than does glucose (8) and, thus, should be a more efficient source of energy, this slight advantage, when considered in conjunction with the relatively small amount of lactose included, certainly can not account for the differences obtained. Lactose, or specifically its constituent galactose, has been credited with increasing the utilization of fat (13). Lactose itself was reported to be antirachitic and to favor calcium metabolism in children as well as laboratory animals (6, 10, 11). By promoting an acid medium, lactose favors the absorption of both calcium and phosphorus, although Robinson *et al.* (14) expressed the belief that acidity was not the only factor. They suggested that lactose or the lactate ion may exert a specific action in facilitating passage of calcium into the blood. It is doubtful

that either calcium or phosphorus was a limiting factor in this study because the synthetic rations were high in these elements. The improbability of such a limitation is further indicated by the fact that the blood plasma levels of these elements were not affected adversely during the experiment. It was reported in the early 1900's that lactose exerts its beneficial influence by favoring desirable bacterial forms in the intestine (9). Perhaps this might be advanced as the logical explanation of the results in this experiment. Even so, it fails to explain the differences between the *KL* and *SL* groups of this experiment and the *L* group of the previous experiment. It would appear that lactose favorably influences the utilization of certain carbohydrates, as well as the utilization of fat. Regardless of the theoretical explanation, these results provide a basis for the common practice of including dry whey or non-fat dry milk solids in calf starters.

#### SUMMARY

Nine neonatal calves were allotted to three experimental groups and fed rations consisting of synthetic milks which varied only in the carbohydrate component.

Calves receiving corn syrup plus lactose (*KL*) gained an average of 28.33 lb., those receiving starch plus lactose (*SL*) gained an average of 24.67 lb., while calves on starch (*S*) averaged only 14.00 lb. gain in 31 days. The efficiency of feed utilization, expressed as pounds of gain per pound of dry matter consumed, was 0.487, 0.412 and 0.204 for the *KL*, *SL* and *S* groups, respectively.

Serial blood samples were collected before a test meal and at 0.25, 0.5, 1, 2, 4, 6 and 8 hr. after feeding and analyzed for blood sugar. The blood sugar level rose rapidly after the ingestion of glucose, lactose or corn syrup, with the maximum concentration at 4, 4 and 1 hr. respectively, after feeding.

Following starch ingestion there was no change in blood sugar the first 4 hr. and only a moderate increase at 6 and 8 hr.

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## THE DIGESTION OF RUMEN MICROORGANISMS BY THE HOST ANIMALS

W. D. POUNDEN,<sup>1</sup> L. C. FERGUSON,<sup>2</sup> AND J. W. HIBBS<sup>3</sup>

Complete utilization of the nutrients synthesized in the rumen by the microflora and microfauna presupposes the later disintegration of the microorganisms in order that the products incorporated in their cells may be absorbed by the host animal. The concept that the rumen microorganisms are digested by the host animals after passage from the forestomachs is quite generally accepted (3). Hastings (9) states that ruminants live to a large extent on protozoa and bacteria which are constantly carried to the true stomach, killed and digested. On the other hand, McNaught and Smith (13) cite Köhler (12) as being of the opinion, based on comparative direct bacterial counts between rumen and intestinal contents, that very little protein in bacterial form is available to cattle. One of the principal details which support the supposition that rumen bacteria are later digested by the host animal is that ruminants can utilize non-protein nitrogen such as urea (4, 8, 13, 14 and 19). McNaught and Smith (13) consider that it would be almost impossible to explain this ability that ruminants possess for utilizing non-protein nitrogen as compared to other livestock such as pigs, rats and poultry, unless the conversion is accomplished by rumen microorganisms which later release their products to the host.

Johnson *et al.* (11) mentioned the possibility that the bacteria utilize products like urea, that the bacteria are later digested by protozoa and these, in turn, are digested by the host animal. Ciliates apparently are destroyed during their passage through the digestive tract as they disappear in the abomasum (1, 2, 6, 18). Baker (2) also has shown that they are digested by peptic and tryptic extracts.

According to Mangold, as cited by Johnson *et al.* (11), ruminants obtain much protein through their digestion of the infusoria which pass along from the rumen. However, the presence of protozoa apparently is not essential to ruminants (5), even for the utilization of urea (11). Hastings (9) interprets this to indicate that bacterial protein is as available to the host as that from protozoa. However, the protein derived from bacterial protein may be somewhat less easily digested by the host than that from protozoa (11, 13).

Baker (2) observed that iodophile bacteria of the rumen, although not affected by gastric secretions, are inconspicuous and present only in limited numbers in the caecum and feces of cattle and sheep and that partially digested microorganisms frequently are seen in caecal contents. The breakdown and disappearance, by the time the materials reached the caecum, of the strongly iodo-

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<sup>1</sup> Present address: Bureau of Animal Industry, Beltsville, Md.

<sup>2</sup> Department of Bacteriology, Ohio State University.

<sup>3</sup> Department of Dairy Industry, Ohio Agricultural Experiment Station.

phile *Oscillospira* organisms which were prevalent in the contents of a sheep's rumen was reported by Baker and Harriess (3). They further mentioned the observation of residues of structural cellulose with enzymic cavities which lacked the bacteria which had produced them as the bacteria had been digested out. Autolysis of iodophile bacteria apparently occurs, according to Baker (2) who demonstrated it by using incubation in the presence of toluene. Large coccoids, morphologically similar to those seen in rumens, were observed by Pounden and Hibbs (15) in the feces of six out of seven samples from cows on mixed rations and in those from seven calves on hay and milk diets.

#### METHODS

It seemed possible that further information might be obtained regarding the fate of rumen bacteria as they progress down the digestive tract by investigating the presence of morphologically identifiable types in various sections or areas of the tract. The method would be somewhat similar to that mentioned by Baker and Harriess (3) for *Oscillospira*. Use was made of the four previously described bacteria which were characteristically found in the rumens of cattle ingesting an adequate proportion of roughage and which were utilized as "indicators" in determining the presence or absence of usual rumen flora in young calves (15, 16). The first consisted of large Gram-positive coccoids which previously were designated as composing hay flora group I. The second ones were the large cigar-shaped Gram-negative bacteria (probably *Oscillospira*). Small Gram-negative rods which tended to form in flat rectangular groups suggestive of window panes and large, thick Gram-positive rods frequently present in pairs were the remaining two used in this study. The latter three are the microflora which make up the previously described hay flora group II.

Gram-stained smears were made from the contents of the rumen and various other parts of the digestive tracts of cows and calves at slaughter. In order to remove the coarser materials, all samples were strained through a single layer of cheese cloth before the smears were prepared. Similar stained smears were made from samples of the contents of various parts of the tract to which rumen contents, particularly rich in the cigar-shaped rods, were added. Smears were made and repeated later to determine if changes occurred in the bacterial "indicators" following incubation of the samples at 37° C. or when kept under refrigeration.

Samples from all parts of the tracts were not always available, partly because some results are included which were obtained during the course of other studies in which only limited preparations were made. An attempt was made to estimate the relative concentrations of these organisms which were present in each preparation for purposes of comparison. Ratings between one and four were assigned on this basis.

#### RESULTS

It was noticed that smears made from abomasal samples sometimes would appear to contain greater concentrations of bacteria than did the rumen. The absorption of water just before passage of food into the true stomach, such as

was referred to by Savage and McCay (17), would be a possible explanation for this change. It also was noted that bacteria tended to be limited in numbers in the duodenum, to increase progressively in concentration down the tract and to become very numerous in the large intestines.

*Large coccoids.* Comparisons were made between 26 freshly obtained rumen samples in which these coccoids were present and samples from other parts of the tract. In seven instances, samples were available from the abomasum, and in all seven these organisms were present. They also were present in all 13 of the small intestine samples and in 22 of the 25 large intestine samples. Similar results were obtained for the few samples of abomasum and small and large intestinal contents which were refrigerated or incubated. The results are tabulated in table 1. Thus, it would appear that to quite an extent, these large

TABLE 1

*The relative concentration of large coccoids in various parts of the intestinal tract of bovines*

Animal	Rumen	Abomasum	Duodenum	Jejunum	Ileum	Caecum	Colon
Cow 600 J	1 <sup>a</sup>	1	1	1	1	2	3
Cow 750 H	2	2	2		2	1	
Cow 743 H	2	1			1	2	
Cow 851 H	2	2			2	2	
Cow 913 J	2	2			2	1	
Cow H	1	1					
Calf 1022 J	1	2	2	2	2	2	
Calf 1022 J + 24 hr. incubation	1	2	1	1	2		
Cow H + 24 hr. incubation	1	1					
Calf 1022 J + 24 hr. refrigeration	1	2					
Calf 885 J + 24 hr. refrigeration	1	1	1	1	1	1	1
Other calves:							
Present in rumen = 18;							
also present in caecum = 15							

<sup>a</sup> Rating: 1 = few; 2 = moderate numbers; 3 = many; 4 = masses.

coccoids are not sufficiently affected by passage through the digestive tract to render their nutrient content available to the host animals unless possibly when predigested by protozoa.

*Very large cigar-shaped rods.* The results obtained (table 2) agreed with the findings mentioned by Baker and Harriss (3) for *Oscillospira*. These large bacteria, although present in the rumens, were absent in all smears, freshly prepared at slaughter, from the contents of other parts. Included were one abomasal sample and two small and seven large intestinal samples. These organisms also were added to abomasal and intestinal samples by use of rumen material containing large numbers of them. They disappeared from the six abomasal and three small intestinal samples. This occurred in approximately 1 hr. at room temperature in the case of five of the abomasal samples, while the one remaining abomasal and the three small intestinal samples were not examined until after 24 hr. refrigeration. These bacteria still were visible in the two samples, one each from the caecum and colon, which were kept refrigerated for 24 hr. after being added.



These large cigar-shaped organisms evidently are destroyed by contact with abomasal and small intestine contents but not by the contents of the large intestines, at least under conditions of refrigeration. Under natural conditions,

TABLE 2

*The relative concentration of large cigar-shaped bacteria in various parts of the intestinal tract of bovines*

Animal	Rumen	Abomasum	Duodenum	Jejunum	Ileum	Caecum	Colon
913 J	2 <sup>a</sup>	0	0		0	0	0
913 J + added bacteria + 1 hr. room temperature	1	0					
750 H + added bacteria + 1 hr. room temperature	1	0					
743 H + added bacteria + 1 hr. room temperature	1	0					
851 H + added bacteria + 1 hr. room temperature	1	0					
1022 J + added bacteria + 1 hr. room temperature	1	0					
600 J + added bacteria + 24 hr. refrigeration	4	0	0	0	0	2	3
Other calves:							
Present in rumen, but absent in caecum = 6							

<sup>a</sup> Rating: 1 = few; 2 = moderate numbers; 3 = many; 4 = masses.

they would never, of course, reach these posterior parts of the intestinal tract.

*Small rods in flat rectangular groups.* Groups of these organisms were readily detected in smears of seven abomasal samples, even though not visible in one of the rumen samples collected at the same time (table 3). They were observed in eight of the possible eleven samples from the small intestines of ani-

TABLE 3

*The relative concentration of small rods formed in flat rectangular groups in various parts of the intestinal tract of bovines*

Animal	Rumen	Abomasum	Duodenum	Jejunum	Ileum	Caecum	Colon
600 J	2 <sup>a</sup>	2	1	1	1	2	1
750 H	0	1	0		0	1	
743 H	2	2			1	1	
851 H	3	2			2	2	
913 H	1	2			1	1	
H	2	2					
1022 J	1	1	0	0	0	0	
1022 J + 24 hr. incubation	0	1					
H + 24 hr. incubation	1	1					
1022 J + 24 hr. refrigeration	1	1					
885 J + 24 hr. refrigeration	1	1			1	1	
Other calves:							
Present in rumen = 4; but absent in caecum = 3							

<sup>a</sup> Rating: 1 = few; 2 = moderate numbers; 3 = many; 4 = masses.

mals which had these bacteria in their rumens. These organisms were present in six samples from the large intestines of similar animals. Unfortunately, few

organisms were present in the rumen of one animal from which were obtained three small intestinal samples that lacked these bacteria along with one similar large intestinal one. Consequently, it is possible that the concentration was too low to permit their detection. Refrigeration and incubation both failed to change the results in the few instances when they were used.

It was possible, therefore, to observe these organisms in samples from the abomasum as well as the intestines. In samples from the large intestine, and sometimes the ileum, groups of these organisms appeared to be breaking apart and stained irregularly. In previous examinations these bacteria were not visible in smears made of fecal samples from both cows and calves. They were not established in the rumen of a calf into which were placed feces from another calf which had received several rumen inoculations with cud materials (15). Thus, it is probable that these bacteria, although visible in samples from the caecum and colon, are in process of disintegration in these organs.

*Large thick rods.* These organisms were readily observed in the seven abomasal samples from animals which also had them in their rumens (table 4).

TABLE 4  
*The relative concentration of thick square-ended rods in various parts of the intestinal tract of bovines*

Animal	Rumen	Abomasum	Duodenum	Jejunum	Ileum	Caecum	Colon
600 J	1 <sup>a</sup>	1	1	0	0	1	0
750 H	1	1	1		1	1	
743 H	2	2			1	1	
851 H	1	2			2	1	
913 J	2	2			2	1	
H	2	2					
1022 J	1	1	0	0	0	0	
H + 24 hr. incubation	2	2					
1022 J + 24 hr. incubation	1	1					
1022 J + 24 hr. refrigeration	1	1					

Other calves:

Present in rumen,  
but absent in caecum = 9

<sup>a</sup> Rating: 1 = few; 2 = moderate numbers; 3 = many; 4 = masses.

They were detectable in six out of eleven small intestine examinations and in five out of sixteen large intestine trials. They were not observed in any of the caecal smears from ten calves which had some present in their rumens. This may indicate a difference between calves and cows. However, the smallness of the numbers available to pass through, coupled with the density of bacteria in caecal samples, may have been responsible for this difference in results. Nonetheless, in one caecal sample from a cow, they were observed to be few and to stain in an irregular manner as though decomposing. It also is noticeable that they were not observed during previous investigations in fecal samples from seven cows and seven calves even though some were known to be present in the rumens of the latter (15). Neither were they established in the rumen of a calf which received, per stomach tube, feces from a calf whose rumen contained them. In the few instances in which a check was made, these organisms

were not visibly affected by abomasal fluids during either incubation or refrigeration for 24 hr. It would seem, therefore, that these thick rods may disintegrate in the posterior parts of the tract.

*Protozoa.* Even though rumen samples from the slaughtered animals used in the above experiments frequently had large numbers of protozoa, these were noticeably absent from abomasal samples. To four abomasal samples which had been held in refrigeration over night, rumen fluid containing large numbers of ciliates was added in the proportion of three parts of the former to one part of the latter. The mixtures were held at room temperature. Motility of all but a few ciliates was halted almost immediately. After 45 min., the vast majority of the ciliates had collapsed and disintegrated or had become swollen and appeared to be ready to disintegrate. The swelling of the protozoa probably occurred prior to the disorganization phenomena mentioned by Baker (1), during which he says the cytoplasm shrinks, the meganucleus becomes deformed and the skeletal rods dissociate.

#### DISCUSSION

The results of this study are far too limited to permit any deductions regarding rumen microflora in general. However, based on the observations made on these four bacterial types, it would be logical to surmise that some organisms undergo rapid disintegration shortly after encountering abomasal fluids. Others manage to hold their form until they reach the more posterior parts of the digestive tract, while still others can withstand all the post-ruminal digestive activities of the animals. A possible means whereby microflora of the latter type could become of value to the host would be through predigestion by the protozoa.

It has been observed that the caecal flora of mice will change greatly depending on the feeds eaten (7), and high milk diets have considerable influence in this respect on rats (10). This situation also could quite conceivably apply to calves. In this way differences in the feeds ingested might possibly result indirectly in differences in the bacterial enzymes produced in the caecum and in the ability of this organ to disintegrate the rumen bacteria which enter.

#### SUMMARY

Observations were conducted on samples from various parts of the digestive tract of cattle for the presence of four types of bacteria characteristically present in rumen samples from cattle. Evidence was being sought regarding the availability of bacterial products to the host.

The first of the four organisms were larger coccoids. Some of them were observed in all parts of the digestive tract, although sometimes apparently in less concentration in the more posterior parts of the large intestine. The second, which were large cigar-shaped organisms, disappeared in the abomasum and were missing in the remainder of the tract. On addition to samples of the contents of the abomasum and small intestines, they were destroyed but not so by those samples from the large intestine.

The third type was composed of small rods which formed in flat rectangular

groups. These appeared to disintegrate gradually as they reached the posterior parts of the tract, although some were observed in samples from the caecum and colon. The same apparently was true of the bacteria of the fourth group, which were thick square-ended rods.

The destruction of protozoa by abomasal fluids, reported by others, was confirmed in this study.

It is concluded from the data, although somewhat limited, that the ultimate fate of rumen microorganisms varies between the extremes of complete destruction in the abomasum to passage entirely through the digestive tract of the host.

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# THE EFFECTS AND INTERRELATIONSHIP OF COPPER, IRON AND PASTEURIZING TEMPERATURE ON THE STABILITY OF ASCORBIC ACID ADDED TO SKIMMILK

R. C. STRIBLEY, C. W. NELSON, JR., ROBERT E. CLARK  
AND F. W. BERNHART

*Research and Development Dept., Nutritional Division, Wyeth, Inc.,  
Mason, Michigan*

The increasing use of synthetic ascorbic acid in milk and milk products places more emphasis on knowledge of the factors affecting its stability, and a wider use of nutritionally significant quantities of iron and copper in milk products has intensified the problem of metal catalysis of oxidation.

The catalysis of ascorbic acid oxidation by copper is well known. Barron and DeMeio (2) and Marston (15) concluded that copper but not iron catalyzed the oxidation of ascorbic acid, but that iron increased the catalytic power of copper and that in all except very highly purified solutions copper was present in sufficient quantity to make the iron appear to be independently catalytic. Because of the natural occurrence of iron and copper in milk, the effects of the two metals cannot be studied separately at the zero level of either and for practical purposes they both may be considered oxidation catalysts.

In studies on the stability of ascorbic acid in milks during processing, storage and use, numerous investigators have noted the destructive effects of added copper (8, 9, 10, 11, 14, 20, 23, 24), the superiority of high temperature pasteurization over holder pasteurization with regard to the losses of ascorbic acid incurred in pasteurization and in subsequent storage and use (11, 14, 16, 23) and the effects of added iron (23). However, there do not appear to be any comprehensive studies on the effect of the concentration of one of the metals upon the activity of the other and on the effects of temperature of pasteurization as related to copper and iron content.

It was the object of this work to study some of the effects and interrelationships of copper, iron and temperature of pasteurization, upon the stability of ascorbic acid added to skimmilk after pasteurization.

## EXPERIMENTAL

Skimmilk was chosen in preference to whole milk because the ascorbic acid oxidation in milk occurs in the aqueous phase and because skimmilk was obtainable from day to day as a more nearly uniform material. The experimental work was designed to determine the comparative effects of the various combinations of the three variables on the stability of ascorbic acid added to the milk after pasteurization during a fixed 16-hr. incubation period under standard experimental conditions. Incubation conditions were chosen to allow sufficient excess of oxygen. The ascorbic acid oxidation in skimmilk under our experi-

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mental conditions has been shown in previous work in this laboratory (4) to approximate a first order reaction as in water solutions (13, 22). The levels of copper used were 0, 0.1, 0.2 and 0.4 ppm.; of iron, 0, 2, 5 and 10 ppm. Temperatures of pasteurization were 50, 65, 75, 85 and 95° C. The control unpasteurized samples were held at 35° C. for parallel times. A 30-min. pasteurizing time was used throughout, except when effect of length of heating time was studied.

*Materials.* Except for the samples milked into glass and contacting only glass throughout the experiments, the milk used was a composite sample of raw skim milk 6 to 18 hr. old, of grade A fluid milk quality obtained daily from a cream separator connected to a 2,000-gal. receiving tank. It had been handled principally in tinned and steel equipment. Milk free from metal contamination was milked into glass, cooled in the receiving flask, centrifuged in glass and the experimental procedure started within 5 hr. of milking time. A composite sample of milks handled entirely in glass contained 0.1 mg. of copper per liter and 0.48 mg. of iron per liter. A composite sample of "receiving plant" milks contained 0.2 mg. of copper per liter and 1.2 mg. of iron per liter.

CP cupric sulfate and U.S.P. ascorbic acid were used. The CP ferrous sulfate used contained 0.01 per cent copper which, at the maximum iron addition of 10 ppm., contributed 0.005 ppm. of copper to the milk.

In the work on milk having no metal contamination, all equipment was Pyrex, washed with nitric acid and finally rinsed with water redistilled from Pyrex. This double-distilled water was used for solutions and for all additions to the milk samples.

*Procedure.* One metal was added as a concentrated solution to a bulk volume of the milk. For each sample, 490 ml. of this milk then were placed in a 1000 ml. Erlenmeyer flask and, if both metals were being investigated, 5 ml. of a freshly made solution of the other metal were added or 5 ml. of water were used when only one metal was to be added. The sample was heated immediately in a water bath at the required temperature ( $\pm 1^\circ$  C.) with intermittent shaking. After heating, the sample was cooled rapidly to 35° C. in cold water, water was added to replace that lost by evaporation and the flask tightly stoppered and held in a 35° C. incubator in the dark for about 1 hr. to reach equilibrium conditions. Ascorbic acid at a level of 100 mg. per liter, was added as 5 ml. of a freshly made solution.

Fifty ml. were immediately withdrawn for the zero-hour ascorbic acid determination. Three hundred ml. were discarded, 0.5 ml. of toluene added as a preservative to the remaining 150 ml. and the flask tightly stoppered. The sample was incubated in the dark at 35° C. for 16 hr. and the loss of reduced ascorbic acid determined.

*Methods of assay.* Reduced ascorbic acid was determined by the colorimetric indophenol dye-xylene extraction method of Nelson and Somers (18) with 3 per cent  $\text{HPO}_3$  as an extractant. Since the data are intended primarily to be comparative, assay was made for reduced ascorbic acid only without correction for interfering substances.

Iron was determined by the alpha-alpha' dipyridyl method of the A.O.A.C. (1). Copper was determined by the method of Bendix and Grabensetter (3).

#### RESULTS

*Effects of time of pasteurization.* Figure 1 shows the effects of heating time at several pasteurizing temperatures, on the stability of reduced ascorbic acid added after pasteurization to milk containing 0.1 ppm., added copper.

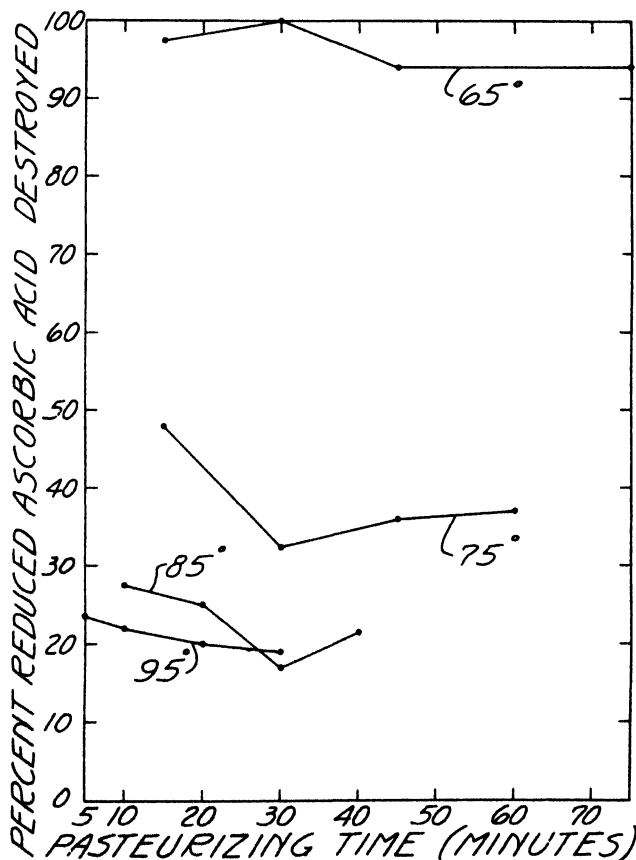


FIG. 1. Effects of time of pasteurization at several temperatures on the stability of reduced ascorbic acid added to skim milk after pasteurization. "Receiving plant" milk containing 0.1 ppm. added copper. Reduced ascorbic acid loss in parallel sample not pasteurized was 35.6%.

At 65° C., maximum loss occurred in samples heated for 30 min. and no significant decrease in loss was effected by heating up to 75 min. At 75 and 85° C., a minimum loss was noted for the samples heated for 30 min., although the increase in loss in samples heated for a longer time was not great. At 95° C.



the ascorbic acid was practically as well retained in samples heated 5 min. as in those heated for a longer time. Except in samples heated at 85 and 95° C., the differences in the effects of temperature of heating were considerably greater than the effect of time of heating at a given temperature.

A 30-min. heating period which represents a compromise between adequate pasteurization at low temperatures and avoidance of excessive heating of the milk at high temperatures was chosen for the remainder of the experiments.

*Effects of temperature of pasteurization in milks of low copper and iron content.* Figure 2 shows the differences in ascorbic acid stability in milk handled in glass and in "receiving plant" milk at various pasteurizing temperatures. The large differences in stability of ascorbic acid added after pasteurization at various temperatures with a minimum stability in samples pasteurized

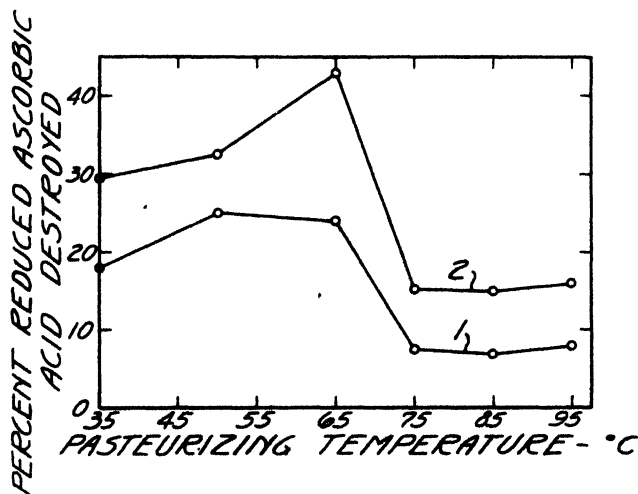


FIG. 2. The effects of pasteurizing temperature on the stability of reduced ascorbic acid added after pasteurization, in: (1) skimmilk handled entirely in glass, average of 2 trials; and (2) "receiving plant" skimmilk, representative trial of 3 trials.

at 65° C. indicate that the heat effects are complex. The destruction of ascorbic acid which occurs in milk handled entirely in glass presumably is due to the copper, iron and other pro-oxidants present in milk as secreted.

The increase in ascorbic acid loss in the "receiving plant" milk, as compared to the milk handled in glass was about constant except for samples pasteurized at 65° C. and probably is due to copper and iron contamination.

*Effects of temperature of pasteurization and added copper.* The effects of added copper at the various temperatures are shown in figure 3. The ascorbic acid losses due to the added copper in the "receiving plant" milk are assumed to be the differences between losses at each copper level and the losses in parallel samples of "receiving plant" milk containing no added copper.

The ascorbic acid losses caused by the added copper varied considerably

with pasteurizing temperature. Significant is the sharp increase in loss in the 65° C. samples. For milk containing up through 0.2 ppm. added copper, pasteurization at from 75 to 95° C. eliminated substantially the ascorbic acid losses caused by the copper. In the unheated samples (35° C.) and at the lower temperatures, the increases in ascorbic acid losses were roughly proportional to the added copper, but the proportionality did not hold at 85 and 95° C.

*Effects of temperature of pasteurization and added iron.* Figure 4 shows the effects of added iron. As with added copper, the greatest ascorbic acid losses occurred in the samples heated at 65° C., although the losses in the 50° C. samples were relatively greater in comparison to the 65° C. samples than with

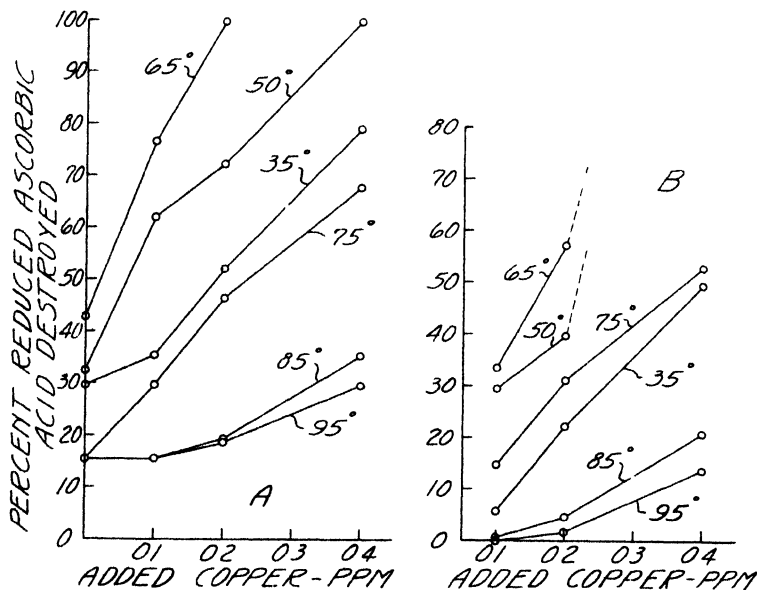


FIG. 3. Effects of pasteurizing temperature on the stability of reduced ascorbic acid added to "receiving plant" skim milk after pasteurization, at several levels of added copper. A, reduced ascorbic acid losses at the various pasteurizing temperatures; B, losses assumed to be due to the added copper. Representative trial of 3 trials. 100% loss points indicate oxidation of all reduced ascorbic acid at an unknown time prior to 16 hr.

copper. As for copper, the loss of ascorbic acid due to the added iron varied with pasteurizing temperature but over a much smaller range, and ascorbic acid losses in the milk pasteurized at the higher temperatures were relatively greater. The 16-hr. ascorbic acid losses in the milk handled in glass and with iron added were much lower than for "receiving plant" milk pasteurized at the same temperature (85° C.), and the losses due to the added iron also were lower.

*Effects of temperature of pasteurization and added copper and iron in combination.* Figure 5 shows the effects of addition of both copper and iron in a

series of samples in which the added copper was 0.2 ppm. and the added iron was varied. Figure 6 shows parallel data at an added copper level of 0.4 ppm. Incomplete data was obtained, as the 16-hr. incubation proved more than enough time for complete oxidation with the higher concentrations of the metals. The losses due to the added iron are assumed to be the differences between the losses at the given iron level and those for samples at the same added copper level but with no added iron. As the copper content was increased, the effect of added iron decreased at 50 and 65° C.; temperatures at which ascorbic acid losses caused by copper were high. Thus, in the 50° C. samples, the ascorbic acid

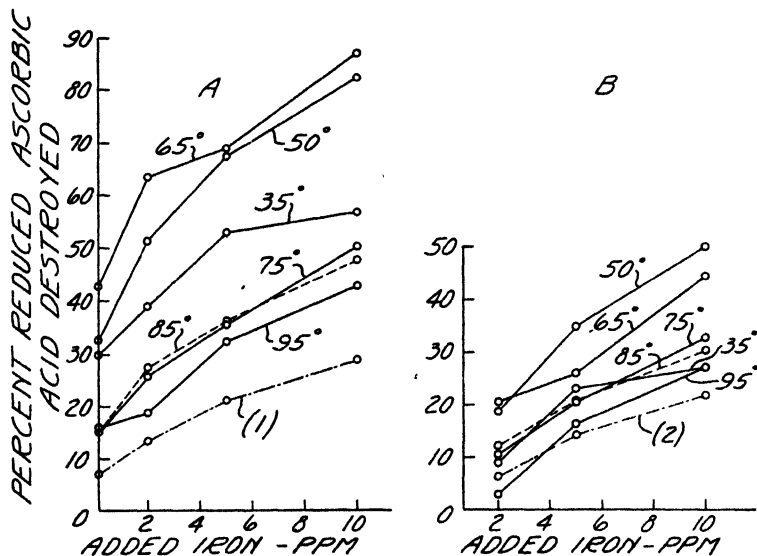


FIG. 4. Effects of pasteurizing temperature on the stability of reduced ascorbic acid added to "receiving plant" skim milk after pasteurization at several levels of added iron: A, reduced ascorbic acid losses at the various pasteurizing temperatures; B, losses assumed to be due to the added iron. (1) Reduced ascorbic acid losses in and (2) losses due to iron added to milk handled in glass pasteurized at 85° C.

losses caused by 5 ppm. of added iron were reduced from 35 to 18 to 0 per cent as the added copper content was increased from 0 ppm. (figure 4) to 0.2 ppm. (figure 5) to 0.4 ppm. (figure 6). However, in the high temperature samples in which the ascorbic acid losses caused by copper were low, the effect of the added iron remained more nearly the same at all three levels of added copper. In the 95° C. samples losses caused by 5 ppm. added iron in the presence of 0, 0.2 and 0.4 ppm. added copper were 14.5, 17 and 17 per cent, respectively.

#### DISCUSSION

Pro-oxidant activity of copper varies widely with the state of combination of the copper, and complexes of the metal have been prepared that show no pro-

oxidant activity. It has been shown that the rate of oxidation of ascorbic acid by oxygen can be used to estimate the concentration of copper having pro-oxidant activity (4, 6, 10, 19, 22).

Therefore, it may be assumed that the rate of ascorbic acid oxidation in skim milk under fixed experimental conditions, including a constant composition of the milk, particularly a constant iron content, is an approximate measure of the pro-oxidant activity of the copper present and that the effects of heating milk on the rate of ascorbic acid oxidation are due to formation of complexes of varying pro-oxidant activities. According to these assumptions, the pro-oxidant activity of copper in milk shows marked variation with pasteurizing temperature.

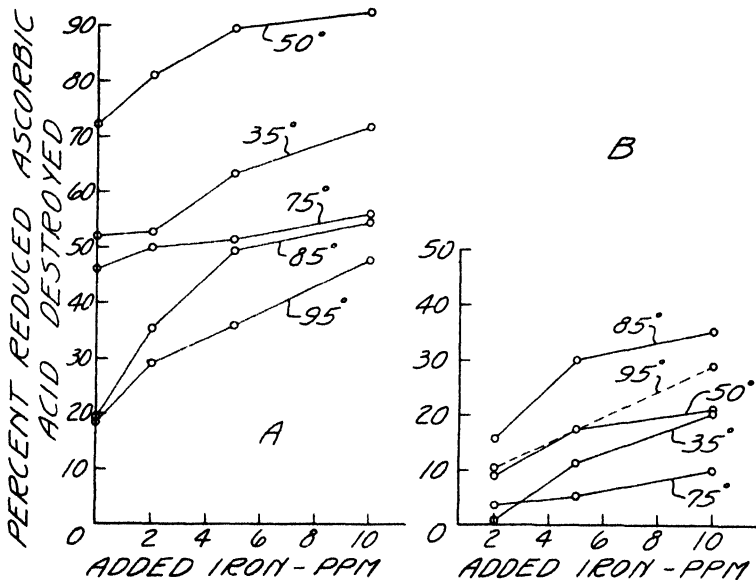


FIG. 5. Effects of pasteurizing temperature on the stability of reduced ascorbic acid added after pasteurization to "receiving plant" skim milk containing 0.2 ppm. added copper, at several levels of added iron: A, reduced ascorbic acid losses at the various pasteurizing temperatures (at 65° C., 100% destruction of the reduced ascorbic acid occurred in all samples); B, losses assumed to be due to the added iron—calculated by subtracting loss for equivalent point in "receiving plant" milk containing 0.2 ppm. added copper and no added iron. 1 trial.

Native, undenatured proteins have metal complexing properties (21) and both the naturally occurring and added copper of unheated milk is present in complex form (4, 17). Greater ascorbic acid losses were found after heating at 65° C. than at 55° C. or for unheated samples (35° C.) so it may be that heating at this intermediate temperature reduces the concentration of naturally occurring copper complexing substances of milk to a minimum; 65° C. is below the point of significant protein denaturation or formation of sulfhydryl groups (5). Heating at temperatures above 65° C. results in a decreased rate of as-

corbic acid oxidation presumably due to copper inactivation. The principal mechanisms of inactivation of copper at higher temperatures probably are the formation of complexes with protein (4, 7) and with sulfhydryl groups produced by heat (4, 5, 11) which are less active than the copper complexes present in unheated milk. Heating 30 min. at 95° C. appears to be sufficient to practically inactivate up through 0.2 ppm. of added copper.

Though the behavior of iron is most correctly expressed in terms of its effect on the catalytic power of copper, as a practical matter, in milk of low copper content, it can be expressed as pro-oxidant activity in a manner allowing com-

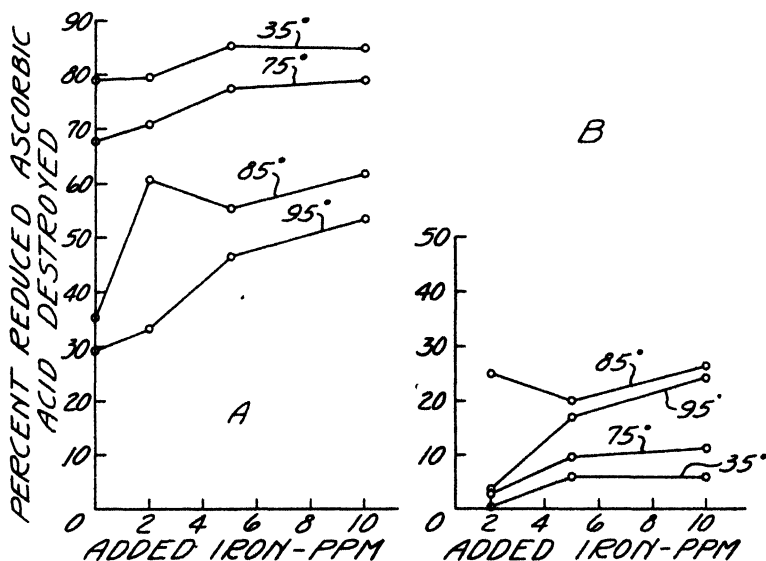


FIG. 6. Effects of pasteurizing temperature on the stability of reduced ascorbic acid added after pasteurization to "receiving plant" skim milk containing 0.4 ppm. added copper, at several levels of added iron: A, reduced ascorbic acid losses at the various pasteurizing temperatures (at 65° C., 100% destruction of the reduced ascorbic acid occurred in all samples); B, losses assumed to be due to the added iron—calculated by subtracting loss for equivalent point in "receiving plant" milk containing 0.4 ppm. added copper and no added iron. 1 trial.

parison with the effects of copper. Figure 7 compares the pro-oxidant effects of copper and iron in the "receiving plant" milk at intermediate added levels of each metal (specifically this data represents the effects of added copper in the presence of about 1.2 mg. per liter of iron and the effects of added iron on the pro-oxidant activity of about 0.2 mg. per liter of copper). The pro-oxidant activity of iron was much less than that of copper. In milk stored at 35° C. the activity of copper was 24 times as great as iron in samples not heated, 55 times as great in samples heated 30 min. at 65° C. and only 4 times as great in the 95° C. samples. The activity of iron varied with temperature of heating but showed only a two-fold change throughout the range of pasteurizing tempera-

tures as against a twenty-fold variation for copper. Iron showed a maximum activity after heating at 50° C. instead of at 65° C. and heating up to 95° C. did not appear to cause inactivation. Successive increments of added iron caused successively less increase in ascorbic acid loss, except in the milk heated at the higher temperatures in which the pro-oxidant copper content was very low (figure 8).

Joslyn and Miller (13) have demonstrated a reduction of the pro-oxidant activity of copper by iron in neutral water solutions of ascorbic acid. In water solutions of pH 7.0 buffered with  $H_3PO_4$ , containing 50 mg. of ascorbic acid per liter, 0.07 ppm. copper and 0.07 ppm. iron, the rate constant for oxidation of ascorbic acid at -1.1° C. was reduced from 5.19 to 1.46 hr.<sup>-1</sup> by increasing the

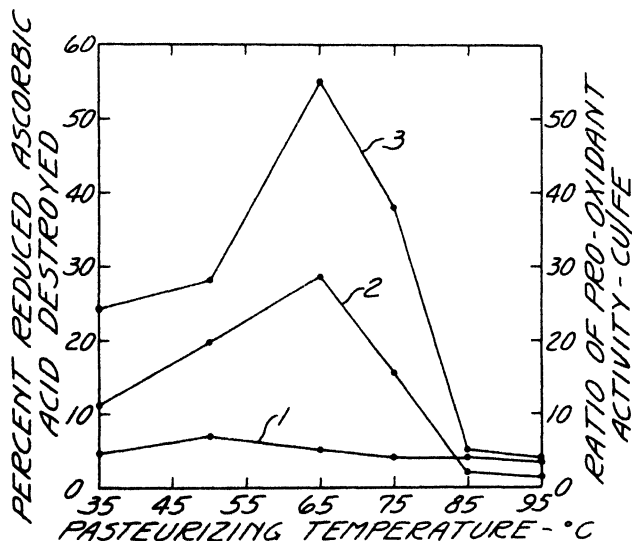


FIG. 7. Comparison of the pro-oxidant activity of copper and iron in milk after 30-min. pasteurization at various temperatures: (1) reduced ascorbic acid loss caused by 1.0 ppm. added iron in "receiving plant" milk containing 5.0 ppm. added iron and no added copper; (2) loss caused by 0.1 ppm. added copper in "receiving plant" milk containing 0.2 ppm. added copper and no added iron; (3) ratio of losses caused by equal concentrations of the metals—copper/iron.

iron content to 11.31 ppm. With milk the data suggest, besides the iron reactions which act to increase the catalytic power of copper, reactions acting to prevent the destruction of ascorbic acid. Within the ranges of copper and iron investigated here, the 16-hr. incubation ascorbic acid loss in milk containing a given amount of pro-oxidant copper was always made greater by the addition of iron. The opposing reaction preventing oxidation of ascorbic acid is illustrated in figure 8 in which the 16-hr. ascorbic acid loss caused by the addition of 0.2 ppm. copper at zero added iron level is compared with the losses caused by this copper in the presence of 2, 5 and 10 ppm. added iron. Here, after heating at 50 and 65° C. where pro-oxidant copper level presumably is high,

the loss caused by the added copper is steadily reduced as the iron content is increased.

As a result of the occurrence of these two opposing reactions the stability of the ascorbic acid could be the same over a range of copper and iron concentrations. This would explain the observation (figure 8) that the increase in ascorbic acid loss caused by addition of 0.2 ppm. copper would (as indicated by the intersection of the curves representing the losses caused by this copper) remain the same in milk containing 0, 2, or 5 ppm. added iron and pasteurized at about 81° C.

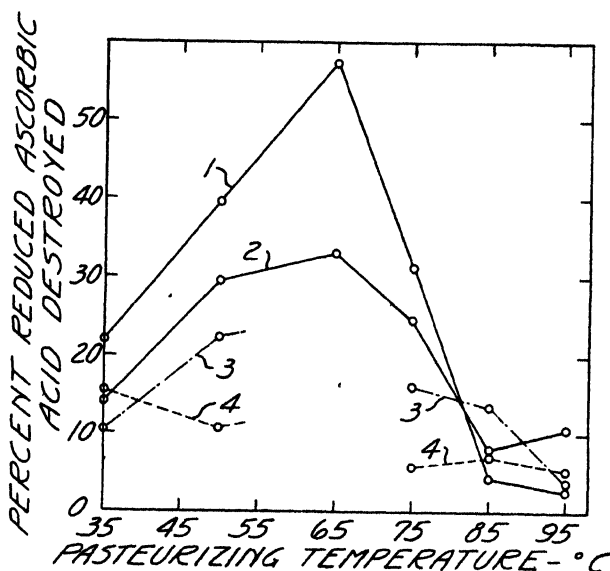


FIG. 8. Effect of added iron on the pro-oxidant activity of added copper as measured by the rates of loss of reduced ascorbic acid added after pasteurization to "receiving plant" skim milk pasteurized at several temperatures. A 16-hr. reduced ascorbic acid loss caused by 0.2 ppm. added copper in the presence of (1) zero added iron; (2) 2 ppm. added iron; (3) 5 ppm. added iron; and (4) 10 ppm. added iron. Calculated by subtracting the ascorbic acid loss occurring at each level of added iron in milk containing no added copper from that occurring with 0.2 ppm. added copper. Data is incomplete at 65° C.

#### SUMMARY

The effects of copper, iron and temperature of pasteurization on the stability of ascorbic acid added to skim milk after pasteurization, and the interrelationship of these variables have been investigated.

1. Pasteurization at temperatures up to 65° C. decreases the stability of ascorbic acid added after pasteurization. Minimum stability occurs at about 65° C. and the ascorbic acid is markedly more stable in milk pasteurized at 75° C. or higher than at 65° C. and in unheated milk.

2. Ascorbic acid added after pasteurization is significantly more stable in

skimmilk handled entirely in glass than it is in grade A fluid quality skimmilk having known copper and iron contamination. The stability of ascorbic acid varies with pasteurizing temperature in the uncontaminated milk in a manner similar to the variation in skimmilk containing added copper and iron, indicating that part of the ascorbic acid losses in uncontaminated milk are caused by the naturally occurring copper and iron.

3. The effect of added copper in the oxidation of ascorbic acid in milk varies widely with pasteurizing temperature. Heating to 65° C. greatly increases the pro-oxidant activity of copper. It then decreases sharply with increases in temperature, and heating to 85 or 95° C. inactivates up through about 0.3 ppm. total copper content.

4. The effect of added iron on the oxidation of ascorbic acid in milk varies with pasteurizing temperature, but over a comparatively smaller range than for copper. The pro-oxidant effect of iron appears to be at a maximum after heating at 50° C. and heating at 95° C. does not decrease the activity greatly.

5. Within the range of added metals investigated, all combinations of added iron and copper caused more rapid ascorbic acid loss than the same level of either metal alone. Under conditions in which the level of pro-oxidant copper presumably was high, however, the ascorbic acid loss caused by each part per million of iron became smaller as the iron content was increased. After heating at 50 and 65° C., the addition of a given amount of copper to milk caused successively smaller increases in ascorbic acid loss rate as the iron content of the milk was increased.

6. The behavior of copper and iron in combination suggests two opposing reactions involving copper, iron and ascorbic acid. The first tends to cause more rapid oxidation of the ascorbic acid, while the second acts to prevent oxidation. The net pro-oxidant effect of the combination of the metals is the resultant of these reactions.

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## THE EFFECT OF RUMEN INOCULATIONS ON THE DIGESTIBILITY OF ROUGHAGES IN YOUNG DAIRY CALVES

H. R. CONRAD,<sup>1</sup> J. W. HIBBS, W. D. POUNDEN<sup>2</sup> AND T. S. SUTTON  
*Department of Dairy Industry, Ohio Agricultural Experiment Station, Wooster*

Conditions in the rumens of cattle fed normal rations are favorable for the growth and multiplication of rumen bacteria and protozoa. It has been shown (9, 10) that most, if not all, of the digestive action in the rumen is due to the functions of these microorganisms.

Several reports (1, 2, 6, 7, 8, 10) have established the fact that the digestibility of fiber by ruminants can be influenced by certain dietary factors. Mangold (10) suggested that the differences in digestibility associated with changes in dietary constituents were related to changes in the gastro-intestinal flora and fauna. This is of interest in view of the recent findings concerning the rumen microbiological picture of young calves by Pounden and Hibbs (11). They reported that under conditions of isolation no more rigid than found on many dairy farms, certain rumen microorganisms characteristically found in normal adult cattle may not become established for many weeks or months. However, when calves were inoculated with bits of cud material from the rumens of normal adult cattle and fed with suitable dry feeds the characteristic rumen microorganisms were established in the rumens of most calves by the time they were 3 wk. of age and in all calves in less than 6 wk. They showed in further investigations (12) that, when the ratio of grain to hay was such that the major part of the rumen ingesta was grain, rumen conditions were unsuitable for either the establishment or growth and multiplication of characteristic indicator microorganisms. When the establishment of characteristic microorganisms was previously accomplished, a decrease in numbers and eventually their disappearance was observed when a high proportion of grain (more than equal parts of grain in proportion to the hay) was included in the ration.

It seemed logical to assume that calves in which characteristic rumen microorganisms are established should be able to digest roughage more efficiently than calves in which these microorganisms are absent or which must depend on a substitute flora and fauna quite different from that normally found in the rumens of adult cattle. Therefore, an experiment using balance trials was conducted in an attempt to determine possible differences in roughage digestion between rumen inoculated and uninoculated calves fed various kinds and amounts of roughage.

### EXPERIMENTAL

The 32 digestion trials to be reported were conducted using ten male Jersey calves.

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<sup>2</sup> Present address: Bureau of Animal Industry, Beltsville, Md.

Hay samples were taken from each bale as it was opened. Hay refused by the calves was carefully recovered at the end of each digestion trial. Both the hay fed and the portion refused were weighed, ground and sampled for chemical analysis.

The calves were bedded with straw ticks made from feed bags. This measure was instituted for the two-fold purpose of preventing the experimental calves from consuming bedding and facilitating quantitative collection of the refused hay.

Feces were collected in triangular bags cut from a rubberized fabric. The feces bags were attached by safety pins to a light canvas calf blanket. The feces bags were changed daily between 8:00 and 10:00 a.m. Wet feces were weighed in the bag and the tare weight subtracted. Immediately, the 24-hr. feces collection was placed in a moisture proof cellophane bag and mixed by kneading for several minutes, depending on the consistency of the feces.

Dry matter was determined on two 200-g. samples of wet feces by oven drying at 100° C. for 24 hr. Two 100-g. samples of hay and pasture were dried similarly for 16 hr. in order to determine the dry matter.

Cellulose was determined by the method of Crampton and Maynard (3). However, digestion of the raw sample was carried out in 50-ml. centrifuge tubes as suggested by Hale *et al.* (5). Considerable foaming was encountered, which necessitated washing down the sides of the flask 5 min. after digestion was started.

The method described by Pounden and Hibbs (11, 13) was used for microbiological examinations of the rumen contents of the calves on the experiment at frequent intervals.

*Experiments with inoculated and uninoculated calves.* Ten male Jersey calves which were dropped during March, April and May in the Ohio Agricultural Experiment Station herd were assigned to either group I or II. Calves assigned to group I were removed from their dams after 1 day and placed in another barn to prevent natural inoculation of the rumen from association with their dams. The dam's colostrum was saved and fed from a pail until the end of the third day, after which the calves were fed whole milk from the Holstein herd at the rate of 0.9 lb. per 10 lb. of live weight, based on the birth weight. This limited amount of milk was fed in order to encourage early roughage consumption. When the calves were removed from the cow, they were provided good quality, third cutting alfalfa hay, free choice. A 4-wk. preliminary period was allowed for calves to begin roughage consumption and attain a somewhat constant daily intake. This plan was altered in the case of the three uninoculated calves, numbers 4, 2 and 8. These calves did not begin eating appreciable amounts of hay until after the fourth, sixth and seventh weeks of age, respectively. At the conclusion of the preliminary period, the first and second digestion trials were conducted for two successive 7-day periods.

Sterilized pails were used for feeding the uninoculated calves. These calves were handled routinely and fed first during all phases of the experiment. Pans

of disinfectant were provided at the entrance of the calf pens for cleaning the attendants' boots.

Calves in group II (inoculated) were treated as group I (uninoculated), except that they were housed without taking special precautions to prevent rumen inoculation through contact with other cattle. The calves in group II also were inoculated with rumen microorganisms by taking small pieces of cud from adult cows in the herd and placing them in the mouths of the calves on the 11th, 16th, and 21st days of age. Extended initial preliminary periods were required for two inoculated calves (5 and 6) since they did not begin eating appreciable amounts of hay until after the fourth week of age.

In a third series of trials, three calves from each group were placed on a ration of alfalfa hay *ad libitum*. A preliminary period of 7 days was followed by a 7-day digestion trial.

Following the trials on alfalfa hay *ad libitum*, two calves from each group were used for a fourth series of trials in which freshly-cut lawn clippings were fed daily. In order to facilitate measuring the amount consumed, the two pairs of calves were housed in the barn. Fresh lawn grass, mostly bluegrass and white clover, was clipped, weighed, sampled and fed to the calves daily. The refused clippings were collected at the end of the week. As in other trials, the fourth digestion period was preceded by a 7-day preliminary period.

*Experiment in which alterations of the rumen microflora and microfauna were made by heavy grain feeding.* According to the evidence of Pounden and Hibbs (12), rations high in grain and low in roughage depressed the numbers of rumen microorganisms which were characteristically associated with relatively high roughage ingestion. Two calves (5 and 6) from the inoculated group were used to test the effect of heavy grain feeding on subsequent hay digestion.

Calf 5 was placed on a ration of one part alfalfa hay and two parts of a commercial pelleted calf starter for 7 days. After the seventh day, during which time 6.7 lb. of starter and 5.0 lb. of hay were eaten, a rumen sample was collected and microscopic examinations revealed that some of the characteristic rumen indicator microorganisms still were present. Hay then was removed from the ration, and the calf was allowed commercial calf starter pellets *ad libitum*. After 1 wk. the calf was found to be free of protozoa and other characteristic rumen microorganisms. At this time, the rumen microflora appeared similar to that usually seen in very young calves and may be compared to an uninoculated calf which had eaten negligible amounts of hay. The ration then was changed to alfalfa hay *ad libitum* in a period of 3 days. A 7-day preliminary period was followed by a digestion trial of equal duration. Microscopic examination of rumen samples revealed that the calf was held free from protozoa and certain indicator hay flora throughout the trial, during which time the calf was isolated to prevent contact with other animals.

Calf 6 was fed 9.7 lb. of the regular herd milking ration (a simple, 14.5 per cent protein mixture of corn, oats, soybean oil meal, bran and salt) and 5.0 lb. of alfalfa hay during a 7-day period, followed by a 7-day period on the simple

grain mixture *ad libitum*; however, examination showed the rumen was not freed of protozoa and hay flora. Since the commercial calf starter had been used successfully in eliminating the protozoa and hay flora from calf 5, it was fed *ad libitum* for 4 days to calf 6. This calf's rumen then was observed to be free of the indicator microorganisms. After 3 days for changing over to alfalfa hay *ad libitum*, a 7-day preliminary and a 7-day collection period followed. However, a rumen sample obtained at the conclusion of the preliminary period revealed that in calf 6, protozoa and hay flora had reappeared. This may have been due to the lack of complete isolation from an inoculated calf in the adjoining pen separated only by iron pipes 4 in. apart, or to incomplete removal of the indicator microorganisms.

#### RESULTS AND DISCUSSION

The influence of rumen inoculations on the digestibility of dry matter, cellulose and protein in calves fed various kinds and amounts of roughage is shown in tables 1 and 2. Inoculated calves (group II) digested a higher percentage of cellulose than the uninoculated controls (group I) during the first and second digestion trials. When the data of these two 7-day trials were combined and treated as one 14-day digestion trial, the differences in digestibility of both cellulose and dry matter were found to be statistically significant (table 1). Thus, rumen inoculations appear to increase digestion of roughage in calves at an early age. However, it is considered to be significant that the increase in digestibility attributed to rumen inoculations during the first two trials was not found in later trials after the calves were taken off milk and were consuming larger amounts of roughage (table 2). This may be explained at least partially on the basis that a substitute microflora, which was able to do a creditable job of cellulose digestion, may have become established in the rumens of calves which were segregated from the natural source of inoculum. The indicator method used was not adequate to measure this possibility.

Since it is known that the digestibility of milk is different from alfalfa hay, a factor which may have affected the percentage of protein and dry matter digested during the first two trials was the constant level of milk consumed for all calves with a variable intake of hay from individual to individual. The calves of group I (uninoculated) had a higher level of hay consumption during the experimental period, since they were at an average older age when placed on trial. The average age at which the daily hay consumption of the calves was sufficiently consistent to begin digestion trials was 8 days earlier for the inoculated calves than for the uninoculated calves.

There are no significant differences in the apparent digestibility of protein by calves with and without characteristic rumen microorganisms. The occurrence of small differences between the inoculated and uninoculated groups indicates that, although the characteristic rumen microorganisms may be involved in the protein digestion, they have little influence on the apparent absorption of protein by the calf. It is interesting to note, however, that the average apparent digestibility of protein differed by approximately 2 per cent in favor of the in-

TABLE 1

*A comparison of the digestibility of dry matter, cellulose and protein in cud-inoculated and uninoculated calves on a ration of alfalfa hay and milk*

Calf no.	Dry matter		Cellulose		Protein	
	Intake	Digested	Intake	Digested	Intake	Digested
	(g.)	(%)	(g.)	(%)	(g.)	(%)
First trial (Calves fed alfalfa hay and milk)						
Uninoculated (group I)						
0	3923	80.47	544.4	59.87	812.6	81.45
1	4651	76.47	811.0	62.83	905.8	74.73
2	2817	83.96	243.0	70.56	516.8	82.84
4	3458	82.00	367.2	53.00	695.7	80.81
8	5112	76.64	688.1	57.61	1086.2	78.83
Mean	3992	79.90	530.7	60.78	803.4	79.73
Inoculated (group II)						
3	3021	83.96	381.2	65.30	665.0	83.44
5	2685	87.99	245.6	69.81	549.1	84.30
6	2964	80.83	380.6	64.81	578.6	76.88
7	2981	85.88	321.6	65.98	641.2	82.36
9	4186	83.80	531.9	68.54	838.1	82.73
Mean	3167	84.49	372.1	66.90	654.4	81.94
Diff.		4.59		6.12		2.21
Second trial (Calves fed alfalfa hay and milk)						
Uninoculated (group I)						
0	4583	76.05	802.0	63.18	867.2	77.56
1	5688	75.04	1051.5	63.21	1120.3	75.16
2	3702	79.89	553.2	58.51	741.1	81.23
4	4344	78.87	704.0	58.22	864.8	80.71
8	5883	73.96	1056.6	63.75	1267.2	79.08
Mean	4850	76.76	834.6	61.34	972.1	78.70
Inoculated (group II)						
3	4085	81.51	702.7	66.43	799.2	81.92
5	4046	80.90	618.0	63.72	801.3	81.48
6	4394	80.61	704.4	60.61	874.7	81.76
7	4421	81.41	800.7	70.75	904.7	82.23
9	5216	79.29	972.6	66.92	888.4	77.07
Mean	4432	80.76	759.6	65.76	853.2	80.99
Diff.		4.00		4.42		2.29
Summary* (trials 1 and 2 combined)						
Group I		78.45		61.24		79.04
Group II		82.29		66.05		81.48
Diff.		3.84*		4.81*		2.44

\* The data were treated statistically according to the procedure outlined by Snedecor (14).

\* Significant at the 5% level.

oculated group in each series of digestion trials. This may have been due to the digestion of protozoa from the rumen and substantiates the evidence of Ferber and Winogradow-Fedorowa (4) who, by estimating the total rumen population and estimating the rate at which protozoa disappear from the rumen, cal-

culated that approximately 2 per cent of the total protein digested daily was furnished in the form of rumen protozoa protoplasm.

As shown in table 3, pronounced decreases in efficiency of digestion followed when a calf's rumen was freed by heavy grain feeding of protozoa and certain bacteria which are associated with a high proportion of hay consumption in relation to grain concentrates. One calf which was held free of characteristic microorganisms after hay feeding was resumed utilized approximately

TABLE 2

*A comparison of the digestibility of dry matter, cellulose and protein in cud-inoculated and uninoculated calves on rations of alfalfa hay or pasture*

Calf no.	Dry matter		Cellulose		Protein	
	Intake	Digested	Intake	Digested	Intake	Digested
	(g.)	(%)	(g.)	(%)	(g.)	(%)
ALFALFA HAY						
Uninoculated						
0	3661	61.09	1135	63.10	617	61.86
1	6287	63.01	1736	61.30	1214	71.53
4	7062	63.59	2032	66.30	1407	69.22
Mean	5670	62.56	1634	63.60	1079	67.54
Inoculated						
3	4691	64.32	1318	62.82	869	69.78
7	5060	63.24	1478	65.61	1201	74.08
9	4358	69.64	1005	60.88	536	67.66
Mean	4703	65.73	1267	63.10	869	70.50
Diff.		3.17		-50		2.96
PASTURE GRASS						
Uninoculated						
1	7367	71.48	2052	81.78	1493	72.52
2	4632	73.46	1260	82.90	1083	74.65
Mean	6000	72.47	1656	82.34	1288	73.58
Inoculated						
3	5422	71.78	1473	82.21	1266	75.22
7	7021	71.74	1879	79.36	1532	76.04
Mean	6222	71.76	1676	80.79	1399	75.63
Diff.		-71		-1.55		2.05

17 per cent less of the available cellulose, 12 per cent less dry matter and 5 per cent less protein in a 7-day trial than did a similarly treated calf 6, in which the characteristic rumen microorganisms reappeared after hay feeding was begun.

Thus, grain concentrates fed *ad libitum* to calves in the form of a commercial calf starter pellets proved to be an effective means of freeing the calf's rumen of protozoa and certain characteristic rumen microorganisms which are associated with hay ingestion. However, a simple, 14.5 per cent protein grain mixture (corn, oats, bran, soybean oil meal and salt) did not completely free

the rumen of calf 6 from the characteristic rumen microorganism in a 7-day period. Perhaps the desired result would have occurred if the grain feeding had been prolonged.

#### SUMMARY AND CONCLUSIONS

A series of balance trials were conducted to determine if calves inoculated with cud material from older cattle and fed rations high in roughage would be able to digest dry matter, cellulose and protein more efficiently than would calves similarly fed but uninoculated.

Five inoculated and five uninoculated Jersey calves first were fed for 14 days on a ration of limited whole milk and alfalfa hay *ad libitum*. During this period, the calves which were inoculated digested a statistically significant higher percentage of the cellulose and dry matter ingested than the calves which were uninoculated. However, this appreciable difference in digestibility between inoculated and uninoculated calves disappeared when some of these calves were later placed on rations of either alfalfa hay or grass clippings only.

TABLE 3

*The effect of the removal of characteristic rumen microorganisms by heavy grain feeding on the digestibility of dry matter, cellulose and protein in calves fed alfalfa hay*

Calf no.	Dry matter		Cellulose		Protein	
	Intake	Digested	Intake	Digested	Intake	Digested
	(g.)	(%)	(g.)	(%)	(g.)	(%)
6 <sup>a</sup>	7128	66.59	1727	64.76	1436	71.63
5 <sup>b</sup>	6504	58.06	1656	53.50	1293	67.42
Difference		8.53		11.26		4.21
% Difference		12.8		17.4		5.9

<sup>a</sup> Rumens freed of characteristic microorganisms which reappeared before the digestion trial began.

<sup>b</sup> Rumens freed of characteristic microorganisms and remained free through the digestion trial.

No statistically significant differences were noted in the apparent digestibility of protein between inoculated and uninoculated calves on any of the rations used. The small, though consistent, difference in protein digestibility of approximately 2 per cent in favor of the inoculated group in each series of trials may be attributed to the digestion of protozoa protoplasm.

A calf freed of all or almost all of the characteristic indicator rumen microorganisms by heavy grain feeding, when placed on a ration of alfalfa hay, showed a marked decrease in digestibility of cellulose in comparison with a similarly treated calf in which the characteristic rumen microorganisms reappeared, possibly due to natural reinoculation.

These results are interpreted to mean that when roughage constitutes the entire dry feed, cud inoculations aided in providing microorganisms which digested cellulose somewhat more efficiently than did microorganisms which became established in the uninoculated calves. The inoculations were observed



to stimulate hay consumption at an earlier age than when no inoculations were given.

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## CONCENTRATED BUTTERMILK IN ICE CREAM<sup>1</sup>

D. H. WILLIAMS, F. E. POTTER AND C. F. HUFNAGEL  
*Bureau of Dairy Industry, Agricultural Research Administration,  
U. S. Department of Agriculture*

In 1949, 2.4 billion lb. of buttermilk containing more than 200 million lb. of milk solids were produced in the United States from the churning of cream. Probably three-fourths of this amount was sour-cream buttermilk, which was utilized as animal feed. Usually only sweet-cream buttermilk is of a quality which makes it acceptable in human food. The object of this investigation was to find some means of increasing and improving the utilization of sweet-cream buttermilk in ice cream.

The use of buttermilk as an optional dairy ingredient is considered one of the controversial points in the proposed Federal Standards of Identity for ice cream and frozen desserts (10). The authors demonstrated that buttermilk does contribute to improvement in whipping ability and flavor of certain ice creams. Whitaker (16) and Josephson and Dahle (6) showed that the inclusion of buttermilk solids in mixes containing butter improved the whipping properties of the mix. The improvement was attributed to the lecithin fraction of the cream. Thurston and Barnhart (14) observed a richer flavor in ice cream containing buttermilk, which they considered was due to the lecithin. The lecithin content of buttermilk has been reported to be four or five times greater than that of the milk from which it is derived (11).

Thomas and Combs (13) demonstrated the use of roller-dried, sweet-cream buttermilk in ice cream and concluded that the whipping rate was greater and the fresh ice cream was drier in appearance than when skimmilk was used. Also, they found an added richness of flavor imparted to the ice cream by the buttermilk. Others (1, 9) have called attention to the potentialities of buttermilk solids for ice cream.

Brown and Janzen (2) prepared skimmilk and buttermilk mixtures in the Vacreator. They concluded that the Vacreator-produced buttermilk concentrates improved the whipping quality of the mix and that they were preferable to dried skimmilk for use in ice cream.

Combs (3), in experimenting with roller-dried milks, reported making ice cream of excellent quality using sweet-cream buttermilk as a source of milk-solids-not-fat.

During a study of buttermilk derived from centralizer sour cream, Tracy (15) found that the maximum quantity of solids from condensed buttermilk (acidity 1.05 per cent) that could be used without producing a noticeable effect upon the flavor of ice cream was approximately 1.6 per cent.

Sweetened condensed buttermilk has been mentioned as a suitable ingredient

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of chocolate coating (12), but its use in ice cream does not appear to have been investigated previously.

#### EXPERIMENTAL PROCEDURE AND RESULTS

Sweetened condensed buttermilk was prepared by following the procedure used in the manufacture of sweetened condensed milk. Cane sugar, at the rate of 12.5 to 13.5 lb. per 100 lb. of buttermilk, was added to an equal weight of water, the solution boiled, filtered and drawn into the vacuum pan following the

TABLE 1  
*The composition of fluid and concentrated buttermilk products used*

Product	Milk-solids-not-fat	Fat	Sugar	Total sol
	(%)	(%)	(%)	(%)
Buttermilk	8.97	0.40		9.37
Condensed buttermilk	26.80	0.90		27.70
Sweetened condensed buttermilk	30.78	1.30	42.68	74.76
Spray-dried buttermilk	91.40	4.60		96.00

buttermilk. This mixture then was concentrated to between 72 and 74 per cent total solids, cooled to 86° F., seeded with lactose and cooled with further agitation to 68° F., after which it was stored at a temperature below 60° F. for later use. Plain condensed buttermilk was made by concentrating fresh buttermilk in a vacuum pan to between 27 and 30 per cent total solids. The data in table 1 show the composition of these buttermilk products and of a sample of commercial spray-dried buttermilk.

Attempts were made to use buttermilk from neutralized sour cream. Several

TABLE 2  
*The effect of neutralization of sour cream on the flavor of ice cream containing buttermilk derived from the neutralized cream*

Titratable acidity		Type of neutralizer	Flavor of ice cream
Raw cream	Neutralized cream		
(%)	(%)		
0.19	..	Not neutralized	Good
0.25	0.12	Lime hydrate	Lacked fine flavor
0.30	0.21	Sodium bicarbonate	Slightly neutralized
0.30	0.23	Lime hydrate	Strongly neutralized, unpalatable
0.40	0.20	Lime hydrate	Strongly neutralized, unpalatable

lots of raw 20-per cent cream were allowed to sour at 72° F. until various percentages of titratable acidity were reached. The acidity of each lot of sour cream was adjusted by use of either lime hydrate or sodium bicarbonate as shown in table 2, and the cream was pasteurized by holding at 150° F. for 30 min. After churning, the resulting buttermilk was concentrated as described previously. In a second trial with neutralized buttermilk, fresh 20 per cent cream was churned and the buttermilk was divided into five lots which were allowed to develop five different degrees of titratable acidity ranging between 0.25

and 0.50 per cent. These lots of buttermilk then were neutralized, pasteurized and concentrated to between 25 and 35 per cent total solids.

The sweetened condensed buttermilk was used in making ice cream mix in the same manner as is sweetened condensed skimmilk, which it resembles in both appearance and composition. Sweetened condensed buttermilk furnished all but approximately 2 per cent of the milk-solids-not-fat in the experimental mix. In the case of plain condensed buttermilk prepared from neutralized sour cream, only one-half of the milk-solids-not-fat of the mix, excluding that supplied by the cream, was replaced with buttermilk solids.

The composition of the mixes was 12 per cent fat, 10 per cent milk-solids-not-fat, 15 per cent sugar and 0.25 per cent stabilizer. The materials used were 40 per cent cream, concentrated buttermilk (either plain or sweetened)

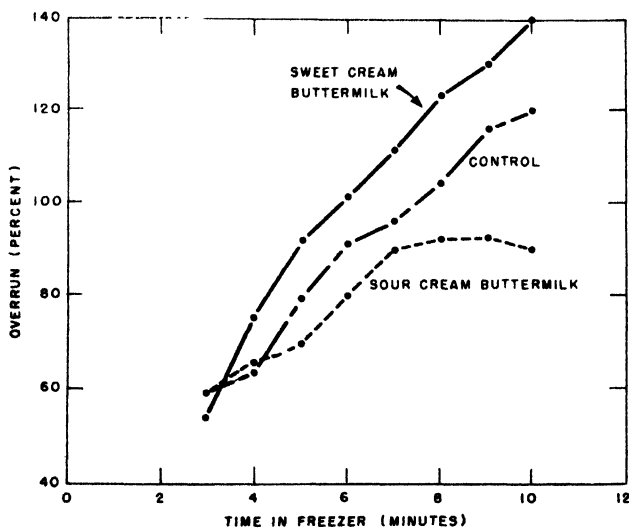


FIG. 1. Comparison of whipping data of mixes containing buttermilk solids from sweet and sour cream with a normal control mix.

and, where needed, plain condensed skimmilk. The small amount of solids-not-fat supplied by the 40 per cent cream was the same in all mixes. Each mix was pasteurized at 160° F. for 30 min., homogenized at 2,500 lb. pressure, cooled to 40° F. and aged 20 hr. During freezing, overrun measurements were taken at 60-sec. intervals, and pint samples of ice cream were drawn from the freezer at 90 per cent overrun. The overrun measurements were used as an index of the whipping ability of the mix (8, 9) and are illustrated in figure 1. Ice cream samples were stored at -10° F. for observation.

Studies were made of the keeping quality of sweetened condensed buttermilk. After storage for 6 mo. at 34° F. there was a slight darkening in color and age-thickening, but the product poured readily and dispersed in the mixing vat satisfactorily. Darkening and age-thickening occurred at 60° F. and these

changes took place more rapidly at 86° F. Figure 2 shows the rate of thickening of a sweetened condensed skimmilk during storage at 86 and 60° F. The data indicate that there probably is little difference between the rates of age-thickening of sweetened condensed milks made from skimmilk and from sweet buttermilk. For best results in retarding age-thickening and for protection against yeasts and spoilage organisms that tolerate sugar, sweetened condensed buttermilk should be manufactured in accordance with the accepted procedures used for sweetened condensed skimmilk. Sweet buttermilk (acidity less than 0.20 per cent) is forwarmed at 180° F. for 10 min., concentrated

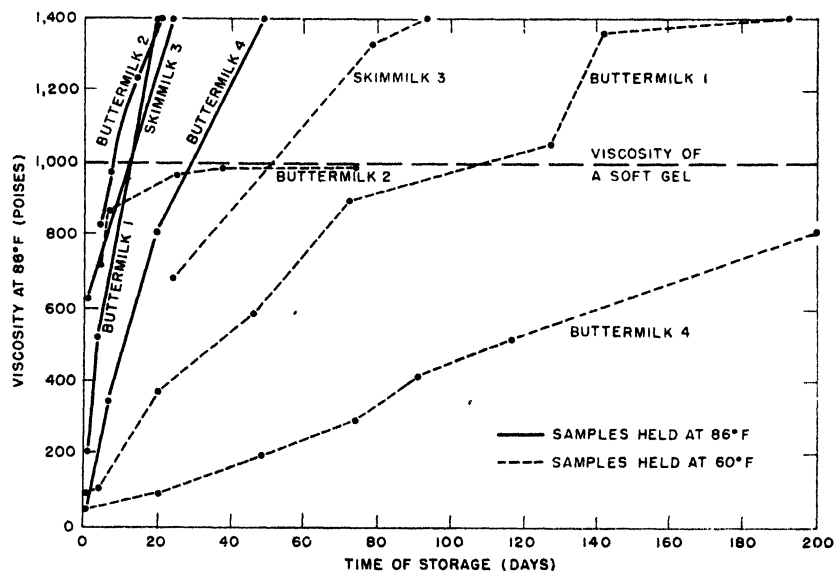


FIG. 2. The effect of storage on the viscosity of sweetened condensed buttermilk and skim milk held at 60 and 86° F. (1) Buttermilk: Acidity 0.16%; preheated at 180° F. for 10 min.; 12.5 lb. sugar added/100 lb. buttermilk; total solids 73.9%. (2) Neutralized sour cream buttermilk: Cream acidity before neutralizing 0.81%; buttermilk acidity at time of concentrating 0.18%; preheated at 162° F. for 15 sec.; 13.5 lb. sugar added/100 lb. buttermilk; total solids 65.73%. (3) Skim milk: Acidity 0.17%; preheated at 180° F. for 10 min.; 12.5 lb. sugar added/100 lb. skim milk; total solids 71.44%. (4) Buttermilk: Acidity 0.17%; preheated at 145° F. for 30 min.; 13 lb. sugar added/100 lb. buttermilk; total solids 74.76%.

with the addition of sugar to give a final sugar-in-water concentration of 62 per cent  $\left( \frac{\text{per cent sugar}}{\text{per cent sugar} + \text{per cent water}} \times 100 \right)$ , cooled to and stored at 60° F.

Solids from plain condensed, sweetened condensed and spray-dried buttermilk, when derived from cream of good quality, produced satisfactory ice cream in concentrations as high as 8 per cent of the mix. By contrast, ice cream in which the sweet-cream buttermilk was replaced by buttermilk solids from cream that had developed more than 0.25 per cent titratable acidity was not palatable. Ice cream was made in which one-half of the normal milk-solids-not-fat was

replaced with buttermilk from cream that had first developed 0.30 per cent titratable acidity. Objectionable off-flavors were distinct and the ice cream was judged unsatisfactory.

A further disadvantage of the neutralized buttermilk was the excessive viscosity which developed in the unsweetened condensed product on the surface cooler. This characteristic, together with undesirable off-flavors, carried over into the mixes and made the utilization of neutralized-cream buttermilk in ice cream commercially impractical. Furthermore, the improved whipping properties observed in mixes containing sweet-buttermilk solids were not obtained when the solids were derived from neutralized sour-cream buttermilk.

Data collected during freezing of the buttermilk mixes in a direct-expansion batch freezer indicate that sweet-cream buttermilk solids increase the whipping ability of the mix. Figure 1 compares the whipping data of a control mix with that of two buttermilk mixes. In this comparison the overrun in the buttermilk ice cream reached 140 per cent in 10 min. and remained there for 15 min. The control mix whipped more slowly and did not reach as high an overrun. The sour buttermilk seriously impaired the whipping ability of the mix.

When mixes containing buttermilk solids were frozen in a continuous freezer, lower air gauge readings were required to obtain the desired overrun than when the control mix, without buttermilk solids, was frozen in the same machine. This is in accordance with the whipping results obtained previously with those mixes on a batch freezer.

Members of a tasting panel reported that the ice cream made with sweet-cream buttermilk had a richer or "creamier" flavor than the control, and, in limited preference tests, the buttermilk ice cream was preferred. The only perceptible difference between the buttermilk ice cream and the control which contained no buttermilk was the sensation of pronounced creaminess produced by the former. Buttermilk ice cream remained satisfactory during storage at  $-10^{\circ}$  F. for 4 mo. Sweetened condensed buttermilk which had been held below  $60^{\circ}$  F. for about 6 mo. was suitable for use in the manufacture of good quality ice cream.

#### SUMMARY AND CONCLUSIONS

Buttermilk solids can be preserved in the form of sweetened condensed buttermilk. This product may be stored for periods up to 90 days at  $60^{\circ}$  F. or at lower temperatures for longer periods without objectionable age-thickening developing.

Only buttermilk of good quality from sweet cream can be used in preparing buttermilk concentrates for ice cream. Buttermilk from unneutralized cream having a titratable acidity at the time of churning in excess of 0.25 per cent is unsuitable for use in ice cream. Buttermilk of unknown history should be examined carefully for off-flavor and excess titratable acidity before it is used in the manufacture of ice cream.

Buttermilk solids from sweet-cream buttermilk are an excellent source of solids-not-fat for ice cream. They are interchangeable with skimmilk solids and may be blended with skimmilk solids to improve the whipping properties

of the mix. Buttermilk solids impart a creaminess to the ice cream not ordinarily obtained with milk solids from more usual sources.

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## FORTY-FIFTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

P. R. ELLSWORTH, *Secretary-Treasurer*

The American Dairy Science Association assembled in Bailey Hall at Cornell University, Ithaca, N. Y. on June 20th, 1950, at 10:00 a.m. K. L. Turk, local chairman, introduced W. I. Myers, Dean, College of Agriculture, Cornell University, who gave the address of welcome. G. M. Trout, ADSA president, was introduced.

### THE PRESIDENT'S MESSAGE

Mr. Chairman, distinguished guests, members of the American Dairy Science Association and visitors:

Thank you, Dean Myers, for those fine words of welcome to the Cornell Campus. Generous as they were, they are excelled by the numerous gestures of hospitality already manifested here amidst these beautiful surroundings. In the brief time we have been in Ithaca, we have noted with appreciation the planning of the various Cornell committees to make us feel at home and to assure us a successful meeting. The large attendance here this morning at the opening session of the 45th annual meeting of the American Dairy Science Association bespeaks the importance of the dairy industry in the nation's agriculture as well as the interest of the Association's members in Cornell University.

As president of the American Dairy Science Association it is my duty to report to you today upon the state of our Association and upon some related matters which I trust shall be of mutual interest.

For the benefit of our younger members, who may not be too familiar with the organization and management of the Association, the constitution, published in the *Journal of Dairy Science*, 26: 788-792, 1943, provides that the business of the American Dairy Science Association shall be directed largely by an elective, short-term, rotating Executive Board. The Executive Board consists of the President, Vice-President, R. B. Becker, Florida; Secretary-Treasurer, P. R. Ellsworth, Ohio; and seven Directors, one of whom is the retiring president. The present Directors are: F. J. Arnold, Iowa; P. R. Elliker, Oregon; H. B. Henderson, Georgia; J. H. Hilton, North Carolina; P. F. Sharp, California; C. W. Turner, Missouri; and W. E. Petersen, Minnesota. In addition, details of the *Journal* are looked after by the Editor, F. E. Nelson, who is also *ex-officio* member of the Executive Board, and associate editors and by the *Journal Management Committee* responsible to the Executive Board. Many

other committees render basic, labor-of-love work for the organization or its sections. Any action taken by the Production, Manufacturing or Extension Sections must be passed on favorably by the Executive Board before the action truly represents the official position of the American Dairy Science Association.

For several years the subject matter of the program of our annual meeting has been assigned to three general-interest groups: the Extension, Production and Manufacturing Sections. At one time an economics section existed, but was short lived. Considerable interest has been manifested during the past year in the reestablishment of such a section. The feasibility of creating an



G. MALCOLM TROUT

economic section should not be treated lightly, for it is becoming more evident daily that marketing is a vital phase of our industry and worthy of more attention from our members. Our host institution, Cornell University, is one of the institutions which has given much attention to dairy marketing and has made notable contributions along this line.



Considering the size of the industry and its relationship to agriculture, the membership of our Association is not large, numbering slightly above 1700 plus approximately 850 student affiliates. In addition, the Journal is mailed to about 1200 subscribers. These figures for 1950 compare favorably with those of 1948 and 1949. The financial picture is healthy but not as gratifying as that of membership. Despite a marked increase in subscription price and a slight increase in membership dues last year it was necessary to dip into the reserve fund to keep operating. This could not continue. Thus, it will not come as a surprise to you to learn that the Executive Board in session yesterday, through necessity to keep the Association solvent, voted to raise membership dues to \$8.00, effective January 1, 1951.

The Journal of Dairy Science is the window of our Association. The scientific world sees the American Dairy Science Association and its activities through the Journal. We are rated as the Journal is rated. It has been a source of pride to hear words of praise from our foreign dairy scientists about the Journal of Dairy Science and to know the esteem which they hold for our Association.

To the credit of the Association and to the editors themselves, there have been but four editors of the Journal in the 33 yr. of its existence, namely, Professor J. H. Frandsen, Dr. A. C. Dahlberg, Dr. T. S. Sutton and our present editor, Dr. F. E. Nelson. The Journal today reflects their abilities and efforts. But editors in themselves do not make journals. Their immediate task is that of editing—not writing. All the editing in the world will not produce a journal unless suitable, original material is submitted.

Many of our members belong to more than one technical organization each of which has a publication of its own that competes more or less with our Journal for original articles. Naturally, authors wish to publish their research conclusions where they will be read by the most people in that particular field of interest. Nevertheless, it must not be overlooked that the wide reader interest of our Association members in dairy nutrition, genetics, bacteriology, chemistry and biology with their applied fields make the Journal of Dairy Science a highly desirable one in which to publish scientific articles. We may be sure that when criticisms are heard to the effect that the Journal is late, that the articles are all production or all manufacturing, or that they cannot be understood, that the fault lies not with the editor or with the Journal Management Committee, but possibly with one of our members who failed to submit a well prepared, original manuscript, with someone who procrastinated in reading and

returning a manuscript or galley proof, or with the reader himself.

Our Journal is more than a scientific journal carrying original articles. Thanks particularly to Dr. Hunziker for his foresight in 1935, it is in part an abstract journal describing briefly and documenting all useable literature pertaining to dairying found in new books, trade magazines and scientific journals throughout the world. Many of our members serve their Association through accepting the responsibility of abstracting certain publications. We need more abstractors in order to give the reader greater journal coverage. Herein lies an opportunity for a young dairy scientist, not only to serve his Association, but also to gain valuable personal benefits and training. Good abstracting takes time but brings compensation; the financial remuneration for such service is only the minor part.

The Journal of Dairy Science, and through it the dairy industry in general could profit by more good review articles. Herein lies a field in which a young dairy scientist might well distinguish himself. In preparing the review a word of caution seems necessary. May the reviewer be thorough and complete in surveying the literature on the subject undertaken so as not to pre-empt the field from another scholar who might have done the job more meritoriously. Many short specific reviews may be written. I cannot conceive of a research worker, or a teacher, either resident or extension, who after having served a few years of apprenticeship has not become obsessed with a certain field of work. In such fields a thorough review article could be written for the mutual interest and benefit of all.

The American Dairy Science Association was the outgrowth of "The Official Dairy Instructor's Association" founded in 1906. The science aspect of the name came as a result of a post-card vote of its members in 1917. While dairy instruction seems to have been the first concept, it was early recognized that good teaching involved knowledge based upon scientific data. Hence, the scientific aspect of the name of our Association came as a logical sequence. Without research, teachers have little to teach; without teachers, results of research are often buried or slow in being adopted. The teacher plays a very important role in dairying today. Through him the student gains his first concepts of the science of the industry and through him those engaged in dairying, production, processing and allied fields are appraised of the latest research. Thus, from the freshman level to the field of so-called adult education the teacher, both resident and extension, wields an influence difficult to measure.

Unfortunately, many of our dairy scientists

engaged in teaching and research look askance at teaching. To be a good teacher does not connote the same as to be a good research worker. To them teaching becomes merely a routine bread-and-butter job. The results of good, effective teaching cannot be measured by the same yardstick as research. Often, only after a lifetime does the role of the teacher manifest itself, whereas recognition comes relatively early in research. However, young dairy scientists cannot be criticized too severely for casting a wary eye toward research and writing to the neglect of effective teaching, since in that way often lies promotion, so long as many of us in college work continue to rate highly the attention-getting factor of research publications and erroneously accept the philosophy that almost anyone who can meet a class can teach.

Our dairy industry needs today as never before trained, versatile, enthusiastic, substantial teachers—teachers who love to teach; teachers who like people; teachers who thrill at the growth in mental stature of their students; teachers who “spark” students with truths that challenge their thinking; teachers who can perceive potential leadership in the freckle-faced, red-headed boy sitting in the front row or trying to conceal himself as far back as possible; teachers with conviction and sustained enthusiasm for their job. Emerson tells us that, “Nothing great was ever achieved without enthusiasm.” Many in this audience this morning owe their leadership today to the “lift” given them by some one good teacher.

Perhaps not many dairy instructors may ever attain the eminence in teaching as did the late Benny Shambaugh of Iowa or Billy Phelps of Yale. Nevertheless, every dairy instructor ought to be able to say with Phelps:

“I do not know that I could make entirely clear to an outsider the pleasure I have in teaching. I had rather earn my living by teaching than in any other way. In my mind, teaching is not merely a life-work, a profession, an occupation, a struggle: It is a passion. I love to teach. I love to teach as a painter loves to paint, as a musician loves to play, as a singer loves to sing, as a strong man rejoices to run a race. Teaching is an art—an art so great and so difficult to master that a man or a woman can spend a long life at it, without realizing much more than his limitations and mistakes, and his distance from the ideal.”

Only by losing oneself in the development of a student may we ourselves develop into a “Mr. Chips.” The beneficial influence of such a teacher in the field of dairy science is beyond price. Many of the younger members of our Association should aspire to become teachers. It is a

noble profession. Henry Brooks Adams points out so challengingly that, “A teacher affects eternity; he can never tell where his influence stops.”

Probably the agricultural world will never know the full worth of dairy extension in this country. As a general rule extension programs have been attacked with a zeal not equalled by other workers. Extension workers have been quick to grasp new ideas and carry them into the field. No one can deny their position of influence and responsibility.

Yet we should consider today the type of dairy extension of tomorrow. This is one of the most perplexing problems with which dairy departments are faced. We have prided ourselves too long in the number of meetings attended, the number of miles driven, or the length of the annual report. Often we are busier than ever and accomplish less. With availability of color photography, flying classrooms, visual aid, sound recording, tabulation machines, radio and television it behooves the extension man to consider seriously how he may better accomplish his work. He may be assured that his clientele, the dairy farmer and processor, through the radio, press, drive-in theater and/or television will have had his interest aroused already in the new findings in dairy research and allied sciences. Therefore, it becomes more imperative today for the extension worker to plan time for studying the literature in order to keep abreast of his field.

Comparing or contrasting the dairy science terminology of 1934, when our Association last met at Cornell, and that of 1950 awakens us with a start to the realization that science marches on and that we must participate actively or be content to sit on the sidelines. The Journal records no mention in 1934, of any discussion or any concern whatsoever for aureomycin, DDT, penicillin, bacteriophage, estrogen, nordihydroguaiaretic acid, geniatics, oxytocin, cobalt deficiency, diluters, thyroxine, *S. faecalis* starters, continuous buttermaking, ring testing, thyroprotein, B<sub>12</sub>, activated flavor, unidentified lactation factors, rumen microbiology, bovine saliva, fast milking, wetting agents, A.P.F. (animal protein factor), quaternary ammonium compounds, yolk-citrate buffer, progesterone, sulfhydryls and/or artificial insemination. Today the effective dairy extension worker must have not only a general concept of such new developments in dairying and related fields of science, but he must be well versed in the research data of the subject of his specialty as well.

While D.H.I.A. records have accomplished their primary purpose in showing the dairymen production abilities of his various cows, they have not yet been analyzed to their fullest extent.

Tabulation of data on I.B.M. cards suggests many possibilities. In fact, Cornell University, our host institution, has taken the leadership in recognizing the possibility of these machines in gleaning further data to aid in greater efficiency of production. With widespread adoption of artificial insemination it becomes imperative to be able to forecast with high correlation the influence of selected sires on the progeny of cows in the mass. To this end, the geneticist with the I.B.M. card system will extend the arm of activity of the extension dairyman.

Our extension dairymen through the county agricultural agents should work more closely with the primary markets for milk and cream. Bias, based upon prejudice and misinformation, stifles progress in quality production. The oldtime idea that the milk plants and creamery managements all are prone to thrive on sharp practices, if not outright dishonesty, and are to be tolerated only because there is no other market should not exist. Carping criticism from or toward the producer or processor merely provides fuel for sensational journalism or unreasoning critics and is helpful to neither branch of our great industry. Let us work more closely with our markets, with the processor and distributor, learning of their problems also, keeping in mind always the ultimate goal of providing the consumer with a good wholesome product at the lowest possible price, commensurate with enabling both the milk producer and manufacturer to operate at a fair profit.

Let us take renewed interest in our dairy extension. It is a work of which we can justly be proud. The accomplishments reveal much: a well managed dairy farm; an efficient herd; a sustained economy; an improved product; a friendly spirit between producer and processor; a confident consumer; a conserved soil fertility; an inspired son; a contented rural family; an abundant living—the very foundations of American democracy.

Probably never before in the history of our Association have there been so many dairy scientists, such a multitude of problems and relatively so little research productivity. The masses of students following World War II required increased teaching staffs and greater teaching loads so that research suffered as a result. Teaching had to be done. As a consequence, research productivity in many of our institutions has been at an all-time low. This situation must be righted and soon. Problems are arising out of proportion to their solution.

Time does not permit the recounting of the major problems in the various branches of the dairy industry, even if we could. We are more concerned today with emphasizing to the young re-

search worker the need of greater research activity and, figuratively speaking, to point out that the research of tomorrow will be started by the young man of today.

Pure versus applied research is not the question. One is void without the other; one appears to be as fundamental as the other; without the first, the second could not exist. Workers in pure research conceive heavy water; those in applied research make the hydrogen bomb. Pioneer research may be carried out in either field.

Scientific truths cannot be discovered without work, without hard work, without obsessive work. Pasteur once said that "chance favors the prepared mind." Fearing the accusation of being facetious, I would venture to add, nevertheless, that chance favors the prepared mind *if the mind is put to work*. Many of the great discoveries of the world were made while the worker was engaged in a phase of work almost unrelated to that for which the worker gained renown. But back of the discovery was an active worker. Pasteur discovered dextro- and levo-tartaric acid, yet his name goes down through the ages associated with bacteriology; Babcock observed metabolic water, yet the world beat a pathway to his door for introducing a relatively simple fat test; Fleming was counting bacteria and gave to the world penicillin; government scientists set out to find a residueless substitute for arsenical sprays for Washington apples and found instead the long-sought cure for nodular disease in Ohio sheep.

These illustrations may seem far fetched, but I do want to emphasize that which every research worker here today knows so well, that a beginning in research must be made; that often in research the goal is never reached; that frequently the paths toward that goal are wandering and disheartening but eventually lead to some fascinating gems the presence of which neither could be predicted nor calculated; and that only by being actively engaged in research can the worker hope to gain that stimulus which enables him to plod on into those unexplored fields where only the active and persistent may hope to delve into Nature's secrets. I believe it was the late Dean Henry of Wisconsin who said, "Nature has always been a guardian of her secrets; they are wrested only by long, laborious, tedious processes."

In general, our remarks along this line represent views not facts. Darwin wrote, "False facts are highly injurious to the progress of science, for they often endure long; but false views if supported by some evidence, do little harm, for everyone takes a salutary pleasure in proving their falseness." Hence, calling these situations to your attention can do no harm; let us hope they may

do some good. The dairy industry is confronted today with many problems from which the American Dairy Science Association and its individual members cannot entirely wash their hands.

The dairy industry, both production and manufacturing, is demanding a different type of training of our college students than that given them in the past. In the future, more emphasis will be placed on fundamentals and less on applications. Teaching of products and cattle will be minimized. Instruction will include, in addition to the basic sciences, business administration, economics, labor relations, psychology and possibly foreign language. Short courses will provide instruction for those who are not qualified or who do not wish to take the basic, 4-yr course. It behooves the American Dairy Science Association to make a thorough study again of the whole dairy curriculum, considering the short course, collegiate, and graduate levels. Our Association had two committees years ago that accomplished some benefits, but further work is needed now. This is not a job for one single institution but for specially appointed committees of our Association working closely with the industry.

The public relations of the dairy industry as a whole are poor. They must be improved and stabilized. The dairy industry loses friends rapidly when big city newspapers carry stories serially of producer-distributor price bickerings giving little consideration to the consumer. Squabbles over inspections, jangles between labor and management, and wranglings, turmoil and distorted facts by an uninformed press over restrictive legislation tend to undermine confidence in the minds of consumers toward dairy products. We may be certain that consumer goodwill will yield more profits to the industry than all the legislative barriers combined. Our Association co-operating with the national breed, product and allied associations could render a real service to the industry by feeding the press regularly popular, sound, dairy science news.

Packaging and merchandising of our products must keep pace with the times. Our Association has led in stressing sanitation and quality, but we all, industry included, have shied at costs associated with good merchandising. In many cases, the consumer has been forced to judge the product by its package—and the package often indicated a cheap product. Recently a check at two Michigan supermarkets revealed seven to eight brands of oleomargarine and one to three brands of butter on display. All the oleomargarine was attractively cartoned with the live yellow color predominating; whereas the butter, in general, was conspicuous by lack of cartons, by its wrinkled

parchment wrapper, misshapen prints and an unwieldy 2-lb. roll. Beside the butter was 15-cent lard similarly packaged. Obviously, in an attempt to merchandise the butter economically, we overlooked eye appeal with the result that sales suffered. Let us remember that price is incidental when buyers yearn for the commodity whether it is butter or Buicks. An industry such as ours cannot survive by catering to nostalgia. The country roll and butter-crock days of merchandising are gone with the cracker barrel. But packaging alone will not suffice; the public must have ready access at all times to good quality products.

The industry will tend to shift from the fat standard. Our industry can no longer be tied to fat alone; each constituent of milk fits into the dairy economy. Non-fat dry milk solids are no more a by-product of milk than is fat or casein. Yet, our whole dairy economy has been built solely around fat and we are loathe to change, but change we must. The discoverer of an accurate, quick, simple total solids test for whole milk will be hailed as the Babcock of tomorrow, for more and more we are going to be concerned with the total solids of milk and not chiefly with fat alone.

Periodically, news releases acclaim some negative quality of whole milk or of its derivatives to the detriment of some branch of the industry. When one branch withers, the tree begins to die. Likewise, when one branch of our industry suffers, the whole industry is affected. Down through the ages the cow has always given whole milk—not a fat-free milk; through the years to come she will continue to do so. The positive qualities of butterfat or other milk constituents and of dairy products must be stressed repeatedly through sound, research-substantiated, regular news releases and attractive advertising. June Dairy Month advertising, beneficial as it is, remains a flash-in-the-pan type of advertising. Sound advertising must be maintained throughout the year. But let us keep in mind that all the advertising in the world will not move continuously inferior products.

The recurring surplus, or overproduction bugaboo can be knocked out. Surpluses are often mere illusions. They disappear quickly in the face of crop failures and of new uses for the product. Consider the citrus fruit industry. Only yesterday oranges growers were faced with surpluses. Creation of frozen, concentrated orange juice not only emancipated the housewife from the tedious job of squeezing oranges but created a shortage of orange groves the extent of which was beyond the wildest dreams of the orange growers themselves. Let us have high quality, basic butter, cheese,

beverage milk, ice cream, evaporated milk, sweetened condensed milk and so on, but may we as dairy scientists neither exclude the possibility of nor be blinded to the needs for special new food products that contain a relatively high content of dairy products. It becomes imperative, if dairy products are to maintain their relative position among the numerous food products found on the shelves and in the refrigerators of our colossal supermarkets, that research leading to the maintenance of quality and the creation of new dairy products be started at once. When such products retain the basic nutritional factors, appeal to the eye and palate, and are promoted intelligently, the looming surplus erroneously thought to be over-production will disappear quickly. We will not need to cry "Wolf" so loudly that Washington may come to the rescue.

The merits of non-fat dry milk solids today remain comparatively unappreciated. Consumers associate these solids with skimmilk, believing that the value portion of milk has been removed in the skimming process. Lactose retails at approximately 75 cents per pound; yet non-fat milk solids, containing 50 to 55 per cent lactose and 30 to 35 per cent of protein made up of the choicest of amino acids, sells for less than 15 cents wholesale. Non-fat dry milk solids might possibly be used more extensively in some existing dairy products and in new dairy products. For years the pediatrician has had prepared milk foods for his prescription; the geriatrician needs such a product badly.

The nutritionists within and without the American Dairy Science Association are to be commended on their researches and admonitions leading to the inclusion of dairy products in the diet. But we must realize that people drink milk and eat dairy products because they like them. Few are concerned with their teeth or health until they are gone. Let us quit giving lip service to quality but insist on palatability of product as well as safety and nutrition. Let us feed more roughages containing higher levels of those factors which tend to stabilize the good flavor of milk. Then may the milk and cream be produced sanitarily and adequately refrigerated so as to retain their original goodness.

Discussion of efficiency of production and management has become stale by repetition. For 50 years we have talked so much, worked so hard and accomplished so little in raising the level of fat production of the cows in the mass. In artificial insemination we have a scientific tool which, if properly employed, will have a marked influence on the level of fat production within a short space of time. But the dairyman must not rest upon the merits of superior germ plasm, upon the service

of proven sires; that is only the beginning. As H. E. Babcock of Ithaca, N. Y., points out, the dairyman himself must be proved. He must know disease control, feeds and feeding and good dairy farm management if he is to get maximum, efficient production even out of superior progeny from mass artificial insemination.

Dairy plant managers are not without their problems. Efficiency, waste disposal, vitamin retention, procurement, processing and distribution problems, important as they are, pale in importance when compared with those of fitting plant operations to the changing conditions brought about by labor. In fact, the dairy processing industry appears to be heading into an industrial revolution. But it will emerge triumphant with more automatic machinery, stationary pipe lines, sealed equipment and continuous operations requiring less labor but that of a highly technically trained, skilled character. Six-day-a-week plant operation is foreseen as common. It is not to be excluded that in the not too distant future the large-city milk supply, as market milk and/or fresh 3 to 1 concentrate, may be processed where labor is not so competitive. As these inevitable major transitions are evolving, the producer-distributor relationships must be such that the changes occur with the least adverse publicity and without hardship to the consumer.

Not in the last half century has there been such a need as now for sound, unselfish, dairy leadership—for leadership that transcends partisan, personal and product lines; for leadership that truly dips into the future while anchored soundly in the present—in brief, a dairy statesman. The dairy industry needs statesmanship. What our industry would give today for a dairy statesman!

In conclusion, may I say briefly that we have a great Association. We all may look with pride at its accomplishments during the nearly half-century of its existence. It is fitting that the opening session of the 45th Annual Meeting of the American Dairy Science Association should be held in Bailey Hall which was named after that idolized patriarch of scientific American agriculture, Liberty Hyde Bailey. At 92 yr. of age, Dr. Bailey looks not backward at his enviable accomplishments in teaching, administration and research, but rather looks forward to the challenging problems and unfinished tasks of the future. Likewise, the members of the American Dairy Science Association and guests are gathered here today, tomorrow and Thursday not to gloat over our accomplishments, but to set our sights to the tasks ahead. We have come to learn of each other's research, to estimate techniques, to discuss the numerous problems and to reassure

ourselves so that we might be better equipped to teach, to investigate and to evaluate the situations which constantly arise in the great field of dairying. May this be a most successful meeting to all.

Chairman Turk then introduced Dr. E. E. Day, former President and Chancellor, Cornell University, as guest speaker. The opening session adjourned at the conclusion of Dr. Day's address.

## BUSINESS MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

*Ithaca, New York, June 22, 1950*

President Trout called the meeting to order at 3:00 p.m. in Room 25, Warren Hall. There were 225 members present.

### REPORT OF THE EXTENSION SECTION

The first session of the Extension section of the 45th annual meeting of the American Dairy Science Association was called to order by Chairman C. W. Reaves of Florida, Tuesday, June 20, 1950, at 1:30 p.m. in Room 140, Warren Hall. Mr. Reaves welcomed the group and made brief comments in regard to the program.

The Chairman called upon Raymond Albrechtsen, New York, to introduce the guest speaker, L. R. Simons, Director of Agricultural Extension, Cornell University, who spoke on the topic, "Effects of Extension in New York."

A short business session included further announcements and committee assignments. The nominating committee appointed included Fred Arnold, Iowa; J. D. Burke, New York; and J. R. Parrish, Alabama.

Papers and demonstrations for integrated county meetings on dairy farm management and dairy subcommittee work in county agricultural planning program were presented and discussed.

Meeting adjourned to the exhibits in Room 101, Warren Hall. Nine exhibits from eight states were reviewed by personnel in charge from respective states. Twenty-two states also had on display bulletins, pamphlets, folders and other literature used as teaching aids in 4-H Dairy Calf Club work. John Foster, Kentucky, served as chairman of the Exhibits Committee.

Wednesday forenoon, June 21, Vice-Chairman Ray Albrechtsen presided. The meeting was called to order at 8:50 a.m. in seminar room, fourth floor, Warren Hall. A symposium on D.H.I.A. organization, operation and record analysis by Dairy Records Committee was conducted by R. G. Connelly, Virginia. Wisconsin presented a paper on types of testing and T. Y. Tanable, Penn State, gave a fine review through the use of colored slides of his work on "The Nature of Reproductive Failures in Dairy Cattle."

A joint session of the production and extension sections was held in Bailey Hall at 1:30 to 5:00 p.m. on Wednesday. G. M. Cairns, Chairman, production section, presided for the symposium

"Grassland Utilization and its Relation to Dairying." Four papers were presented. C. W. Reaves presided for the joint committee reports. These included:

1. *The Program of the Purebred Dairy Cattle Association.* Fred S. Idtse, Secretary, Purebred Dairy Cattle Association. Mr. Idtse gave an historical sketch from its beginning, reviewed its work, problems, policies, aims and achievements, all directed toward a sound constructive dairy herd improvement program.

2. *Breeds Relations Committee.* A. R. Porter, Chairman. Recommended desirable changes in the combined rules and regulations for official testing and registration of animals born as a result of artificial breeding.

3. *Dairy Cattle Health Committee.* No report.

4. *Dairy Cattle Breeding Committee.* Joe S. Taylor, Chairman, reporting, made the following recommendations: (a) New PDCA Rules and Regulations governing artificial breeding. (b) Advisability of encouraging active participation of artificial breeding leaders in American Dairy Science Association programs. (c) The wisdom of an additional service fee if offspring is to be registered was questioned. (d) The need for spelling out the ABC's of genetics for sire selection committees in artificial breeding. (e) Efficiency reports in artificial breeding.

5. *Type Committee.* W. S. Tyler, Secretary, reporting. Recommended program be continued. That respective colleges and universities cooperate with a long-time study in classification work with complete detailed information and classification of their dairy herds yearly.

All committee reports were approved by the joint session.

Thursday, June 22, Chairman Reaves called the meeting to order at 9:15 a.m. Papers were presented on 4-H Club work. Following discussions, the business session was opened by the Chairman.

Committee reports were read, discussed, amended and approved.

The Extension Dairyman Award Committee presented a plan for an Extension Dairyman Award which was approved for submission to the Executive Board of the American Dairy Science Association for action.

The nominating committee named two candidates for Secretary of the Extension Section for 1950-1951. Ivan Parkin of Pennsylvania was elected Secretary.

Chairman Reaves called the final session to order at 1:30 p.m. Papers pertaining to dairy cattle health were presented. The section then adjourned to the business meeting.

Respectfully submitted—RAMER D. LEIGHTON, *Secretary*; C. G. REAVES, *Chairman*; RAYMOND ALBRECHTSEN.

Upon motion duly seconded, the report was accepted.

## REPORT OF THE MANUFACTURING SECTION

The program for the Manufacturing section was carried out as scheduled and published in the May issue of the *Journal of Dairy Science*. A total 49 submitted papers were presented. Papers M8 and M46 were not given.

The business meetings of the sections were held Tuesday, June 20, at 4:30 p.m. and Wednesday, June 21, at 4:00 p.m., with approximately 75 members in attendance at each meeting. Chairman Josephson presided.

Reports of the following standing committees were read and accepted:

### 1. Milk and cream.

#### Subcommittees

(a) Standardization of the acidity test of all dairy products.

(b) Standardization of the Babcock test.

### 2. Dairy by-products.

### 3. Butter.

### 4. Dairy product judging.

### 5. Milk proteins.

The section voted to continue the above committees.

The section voted approval of the amended recommendations of the standing committee on butter and approved the publication of the committee report in appropriate form, subject to approval by the Executive Board, in a suitable journal.

The section commended the Milk Industry Foundation for the plan which provides six annual Leadership Awards to outstanding dairy manufacturing students in the United States and Canada.

The following officers were elected to serve for the coming year: O. F. Garrett, *Secretary*; E. L. Jack, *Vice-Chairman*; and J. H. Hetrick, *Chairman*.

Respectively submitted—D. V. JOSEPHSON, *Chairman*; J. H. HETRICK, *Vice-Chairman*; E. L. JACK, *Secretary*.

Upon motion duly seconded, the report was accepted.

## REPORT OF THE PRODUCTION SECTION

The Production section held four sessions for presentation of technical papers, at which 76 papers were presented. During each session, two sections were run concurrently, with Chairman G. M. Cairns and Vice-Chairman L. O. Gilmore presiding. The authors of the papers are to be commended for having their material well prepared and for keeping well within the prescribed limits in presenting it.

One joint session was held with the Extension section as a symposium on Grassland Utilization and its Relation to Dairying. G. M. Cairns presided.

This symposium was followed by a joint business meeting of the Production and Extension Sections, C. W. Reaves presiding.

The business meeting was called to order at 4:00 p.m., June 21, 1950.

Fred Idtse, *Secretary of the Purebred Dairy Cattle Association*, discussed the program of this association.

The following joint committee reports were read and acted upon:

*Breeds Relations Committee* report read by A. R. Porter, *Chairman*. Moved by Floyd Arnold, seconded by C. L. Blackman that the report be recommended to the Association for acceptance. Passed.

*Breeding Committee* report read by Joe S. Taylor, *Chairman*, who moved that it be recommended to the Association for adoption. Seconded by L. O. Gilmore. Passed.

*Type Committee* report read by W. J. Tyler who moved that it be recommended to the Association for acceptance. Seconded by L. O. Gilmore. Passed.

*Dairy Cattle Health Committee*. Chairman Joseph C. Nageotte stated that the committee had no formal report and asked for suggestions for developing next year's program by the committee.

The joint business meeting of the Production and Extension sections adjourned at 4:55 p.m.

The business meeting of the Production section was called to order at 11:00 a.m., June 22, 1950 by G. M. Cairns, *Chairman*.

I. W. Rupel presented the report of the nominating committee (E. Weaver, L. A. Moore). In keeping with the policy of recent years, they nominated L. O. Gilmore, present Vice-Chairman for Chairman and N. N. Allen, present secretary, for Vice-Chairman, and submitted names of P. A. Kelly and George Hyatt, Jr., for secretary. There were no further nominations. P. M. Reaves moved that the report of the nominating

committee be accepted and that the chairman and vice-chairman be elected by acclamation. Seconded by Fordyce Ely. Motion carried. As a result of a ballot, George Hyatt, Jr., was elected secretary of the Production section.

George Trimberger read the report of the Dairy Cattle Judging Committee, a copy of which is attached.

I. W. Rupel, commenting on the recommendation of admission of teams from two non-land grant schools to the National Collegiate Contests called attention to the large number of schools which offer work in agriculture and the difficulty of appraising their qualifications for participation.

L. L. Rusoff commented on the quality and scope of the dairy work at Southwestern Louisiana School of Agriculture and recommended their approval.

George Trimberger moved that the committee report be accepted and recommended for approval at the General Business Session. Seconded by L. L. Rusoff. Carried.

George Trimberger suggested that it be the policy of the committee to recommend admission or non-admission of any non-land grant schools with action by the Production Section rather than the committee. R. B. Becker and I. W. Rupel offered comments. Dwight Seath moved that admission of any non-land grant schools be decided by action of the Production Section upon recommendation of the Dairy Cattle Judging Committee. Seconded by Fordyce Ely. Motion carried.

J. B. Shepard presented a progress report for the Committee on Pasture Investigations Technique. He called attention to the fact that a final report required incorporation of reports from Pasture Investigation Committees of two cooperating organizations, which can probably be accomplished before the 1951 meetings. He moved that this be accepted as a progress report. C. W. Turner seconded his motion. Carried.

A. R. Porter called attention to the announced policy of the Purebred Dairy Cattle Association of inviting the Chairmen of the Production and Extension Sections, in addition to the Chairman of the Breed Relation and Dairy Cattle Breeding Committees, to participate in their annual meeting.

Meeting adjourned at 11:45 a.m.

Respectfully submitted—G. M. CAIRNS, *Chairman*; LESTER O. GILMORE, *Vice-Chairman*; N. N. ALLEN, *Secretary*.

Upon motion duly seconded, the report was accepted.

## EDITOR'S REPORT

The 12 issues of Volume XXXII of the JOURNAL OF DAIRY SCIENCE printed during 1949 contained 870 pages of original articles, 84 pages of review articles, 8 pages of Association announcements, 17 pages of program for the annual meeting, 20 pages of proceedings of the annual meeting, 36 pages of abstracts of papers presented at the annual meeting, 51 pages of indices, 6 pages of table of contents, 39 pages of membership list, 186 pages of abstracts and 2 pages of miscellaneous. This makes a total of 1,327 pages, exclusive of the advertising sections and blank pages. This is an increase of 24 pages over 1948, but the actual increase in printed material is greater than that because 242 pages of material of a type not previously printed double column and in smaller type were so printed in 1949.

Of the 129 papers printed, 70 were on production subjects, 53 were on manufacturing subjects and 6 were reviews. This represents an increase of 27 manuscripts over 1948. Nineteen manuscripts were rejected during the year, a percentage which is just slightly above that of the past few years. Thirty-nine manuscripts were carried over from 1949 to 1950, many of them in various stages of preparation for printing, this is a reduction of 16 from 1948, indicating a somewhat more current basis of publication. Quite a few manuscripts have been delayed in publication by the slowness of certain authors in getting the manuscripts back to the editorial office following return to authors for a final check before the material went to the printers.

A total of 929 abstracts was printed in 1949, well over double the number printed in 1948 before the Abstracts of Literature Section was reorganized. The double-column organization and the use of a slightly smaller type size have permitted the printing of 113 per cent more abstracts with an increase of only 16 per cent in the number of pages. The type-setting per word costs are almost exactly the same with either form, but the press work, paper, binding and mailing costs are determined by the actual number of pages; thus, a considerable saving has been effected by the new form. Abstracts printed or in the hands of the printers for the first seven issues of 1950 number 550, a slight increase over last year at the same time.

The editor wishes to thank all of those who have assisted in any way in the handling of the affairs of the Journal. Only because of the splendid cooperation which is given by a large number of people is it possible even to publish the Journal, let alone maintain the standards set for the publication. Two men who have served



the Journal and the Association both long and well are retiring as Associate Editors this year; to Dr. O. F. Hunziker and Dr. C. A. Carcy go the thanks of the Journal group and also of the Association. Dr. P. R. Elliker retires as a member of the Journal Management Committee; the Association is fortunate in that he will continue as a member of the editorial group.

As the report of the Secretary-Treasurer shows, the Association is spending more money than is being received, largely because of the current high costs of printing the Journal. Please bear with the reviewers and the editor when suggestions are made for the shortening of manuscripts or abstracts. Apparently, greater brevity of expression is going to be required if dissipation of the operating reserve which was accumulated over a period of years is to be stopped. The editor will do all that he can within the framework of the rules under which he must operate to avoid deficit spending.

Any member who could help by preparing abstracts from one or more journals not now covered in the Abstracts of Literature section is invited to contact the editor. The help of those who can prepare abstracts of papers appearing in foreign-language journals is especially desirable now that such publications are more available. If one or more persons in each institution would assume the responsibility of seeing that a copy of each bulletin or circular reached the editorial office so that a suitable abstract could be prepared, the coverage of dairy literature would be improved considerably.

Respectfully submitted—F. E. NELSON, *Editor*

Upon motion duly seconded, the report was accepted.

## SECRETARY-TREASURER'S REPORT

### MEMBERSHIP.

The following is a summary of our gains and losses for 1949.

Membership, December 31, 1948 .....	1747
Gains:	
New members, 1949 .....	139
Former student affiliates ....	17
Total gain .....	156
Losses:	
Members resigned .....	10
Members delinquent .....	163
Members deceased .....	5
Total loss .....	178
Net membership loss .....	22
Membership, December 31, 1949 .....	1725

This reduction in members is not desirable, but is present, nevertheless, and offers a real challenge to all members of the Association. If each member were to make a point of signing up a new member during 1950, this deficit could be erased and our membership nearly doubled. Letters

written to prospective members by the secretary have netted a large percentage of favorable replies, but more names are needed if this system is to continue to function. Membership in 1949 exceeds the 1947 membership none the less.

### CIRCULATION.

Circulation of the Journal for the year 1949 reached a total of 4,030 by the end of the year, an increase of 146 over 1948 circulation figures. The increased circulation is accounted for by subscribers and student affiliates.

### STUDENT AFFILIATES.

The student affiliate picture is encouraging. The year 1949 saw an increase of 79 over 1948. Since the real purpose of student affiliate membership is to encourage students to assume full membership upon graduation, it becomes the duty of each department chairman to contact the graduating seniors relative to full membership. Letters sent out from the secretary's office to graduates whose names and addresses were furnished by some department chairmen resulted in a most favorable response. Over 50 per cent of those contacted paid 1950 dues for full membership. During 1949 the Virginia Polytechnic Institute Student Branch Certificate was renewed. At this date there are 21 student branches in the country.

### FOREIGN CIRCULATION AND MEMBERSHIP.

Foreign circulation and membership continues to increase. At the present time our Journal goes to 45 foreign countries, reaching both subscribers and our newly acquired foreign members. England and Australia lead in this field with a total of 117 Journals. Several men of science residing in foreign countries have been accepted into membership through the provisions made at the 1949 annual meeting.

### BONDS.

The foresight of the late R. B. Stoltz in purchasing Government Bonds in past years has started to pay off. During 1949 three \$1,000.00 bonds matured and were redeemed. The money resulting therefrom was used to purchase four new \$1,000.00 bonds which are now in the safety deposit box at the bank.

### TEN-YEAR INDEX.

Work is nearing completion on the Ten-Year Index for the Journal and we hope to have it completed sometime during 1950 or early 1951. Mr. Macy, who is doing this work, is to be congratulated on his efforts and devotion to the job.

### ASSOCIATION FINANCE.

During the year 1949 the Association operated at a \$7,962.60 loss. This was expected, since the

die for 1949 was cast at the 1948 meeting where no increase in dues or subscription rates was authorized. With Journal costs constituting 74.7 per cent of the total and with membership dues still not large enough, a raise in dues for 1951 seems an absolute necessity. All other costs of the Association have remained approximately constant and constitute 25.3 per cent of the total.

I wish to take this opportunity to express my sincere thanks to all those members of the Association who have made the past year an interesting and enjoyable one for me through their willingness to work far above the call of duty for the Association's benefit and advancement.

Respectfully submitted—P. R. ELLSWORTH,  
*Secretary-Treasurer.*

Upon motion duly seconded, the report was accepted.

### AUDITING COMMITTEE REPORT

June 8, 1950

To the Executive Board and Members of  
The American Dairy Science Association  
Gentlemen:

On June 8, 1950, Mr. Walter C. Burnham, a Certified Public Accountant, met with the Auditing Committee of the American Dairy Science Association. At that time, Mr. Burnham's report of his audit of the Association's business for 1949 was considered.

Mr. Burnham has made a thorough examination of the records. He has checked the bank statements and examined all the United States Government Bonds. Mr. Burnham has check-tested the inventory of Journals and Twenty-Year Index to assure accuracy of the physical inventory.

The Auditing Committee is satisfied that the financial statement for the year 1949 is correct. The committee wishes to commend Mr. Burnham, the auditor, for his fine work and excellent report. We recommend that the financial statement be accepted by the Executive Board and the members of the American Dairy Science Association.

Respectfully submitted—T. S. SUTTON; C. G. MCBRIDE; FLOYD JOHNSTON, *Chairman.*

Upon motion duly seconded, the report was accepted.

### JOURNAL MANAGEMENT COMMITTEE REPORT

During the past year the Journal Management Committee has authorized the following action:

1. That the proposal of the Journal of Dairy Research for a reciprocal advertising arrangement between that publication and the Journal of Dairy Science be approved, provided our Secretary-Treasurer can arrange an equitable basis for exchange of advertising.

2. That the proposal of our Secretary-Treasurer to reduce the number of copies of the Journal of Dairy Science printed each month from the present 4,400 to 4,200 copies be approved. This proposal was prompted by the fact that the maximum number of Journals mailed out in any 1 mo. in 1949 was 4,028. The surplus of 372 copies, representing more than 9 per cent of total circulation, has created a serious storage problem, and is considerably in excess of anticipated future demand for the Journal.

3. That the Editor and the Secretary-Treasurer investigate the feasibility of incorporating a page in the Journal each month devoted to editorials, Association business, news items, or other items of general interest to Journal readers.

4. That in conformance with the established program for rotating associate editorships, C. A. Carey and O. F. Hunziker be retired, and L. A. Moore and F. J. Doan be appointed as Associate Editors.

5. That the present restriction whereby the Editor of the Journal is not authorized to accept manuscripts from non-members be recuded, with the provision that publication of such manuscripts be subject to approval by the Journal Management Committee.

The Journal Management Committee wishes to express the commendation of the Association membership to the Editor and the entire Editorial Staff for their continued untiring efforts and excellent accomplishments.

Respectfully submitted—P. R. ELLIKER, *Chairman*, G. H. WISF; J. K. LOOSLI.

Upon motion duly seconded, the report was accepted.

### RESOLUTIONS COMMITTEE REPORT

WHEREAS: The Cornell University through its administrative staffs and faculty has made available to the American Dairy Science Association in this its 45th Annual Meeting all needed physical facilities for the meeting, and

WHEREAS: Every possible personal courtesy has been given to members of the Association for their enjoyment and entertainment,

Therefore, be it RESOLVED: That the American Dairy Science Association take this opportunity officially to extend its thanks and appreciation and hereby request the President of this Association to convey by letter this appreciation to Dr. C. W. de Kiewiet, acting president, and to Dr. E. E. Day, former president and chancellor, to Dean W. I. Myers and to Professors K. L. Turk and J. M. Sherman.

WHEREAS: Many commercial and civic organizations have contributed greatly to the success and enjoyment of this 45th annual meeting,

Therefore, be it **RESOLVED**: That the American Dairy Science Association express to these organizations its sincere appreciation.

**WHEREAS**: The Borden Company Foundation again has offered its awards for outstanding research in dairy manufacturing and production.

Therefore, be it **RESOLVED**: That the American Dairy Science Association express to the Borden Company Foundation its sincere appreciation of this evidence of its continued interest in dairy research.

**WHEREAS**: The American Feed Manufacturers Association has seen fit to offer an award for outstanding research in the field of dairy cattle nutrition.

Therefore, be it **RESOLVED**: That the American Dairy Science Association express to the American Feed Manufacturers Association its sincere appreciation for their interest in and encouragement of research in dairy cattle nutrition.

**WHEREAS**: The dairy industry is a most important segment of our agricultural economy and the dairy cow is an important means of converting farm crops into valuable human food, and

**WHEREAS**: The consumption of dairy products is not great enough to utilize all of the constituents of milk now being produced, and

**WHEREAS**: Milk and its products are highly nutritious and are needed in larger amounts in the average diet,

Therefore, be it **RESOLVED**: That the American Dairy Science Association call to the attention of research and educational workers and agencies, both public and private, these facts and urge on them intensified efforts to develop new and improved ways of using milk and the by-products of milk and to bring to the attention of consumers the high nutritional value and the economy of using more dairy products.

**WHEREAS**: Economic and marketing problems are becoming increasingly important in the production, processing and distribution of milk and its products, and

**WHEREAS**: These types of problems have not received the attention of this association that their importance justify,

Therefore, be it **RESOLVED**: That the Executive Board of the American Dairy Science Association consider making provisions for the presentation of material dealing with economic and marketing problems of the dairy industry.

**WHEREAS**: The rapid development of the dairy industry and the ever increasing expansion into new fields of production, manufacture and distribution calls for a highly trained specialized type of worker, and

**WHEREAS**: There is an immediate need to intensify and adopt the training of young people

entering the dairy field to meet the problems of the industry.

Therefore, be it **RESOLVED**: That the Executive Board of the American Dairy Science Association appoint a committee to develop dairy curricula that will more completely meet present day needs.

Respectfully submitted—**GLEN W. VERGERONT**;  
**R. E. HODGSON**; **H. O. HENDERSON**, *Chairman*.

Upon motion duly seconded, the report was accepted.

#### NECROLOGY COMMITTEE REPORT

Your committee regrets to advise that during the current year the following members of the Association have been taken from our midst and have passed on to their final resting place:

*Charles Sterling Trimble*, Dairy Manufacturing Technologist for the Bureau of Dairy Industry, U. S. Department of Agriculture. He passed away in Washington, D.C., on February 21, 1950. He was born in Calhoun County, Iowa, on September 20, 1889. At the time of his death, he was in charge of the Bureau's regulatory work for the inspection of renovated or process butter. Mr. Trimble was graduated from Iowa State College in 1911. For the next few years he was employed in varied capacities in several mid-western creameries. He joined the dairy division of the Bureau of Animal Industry in 1917 as a dairy manufacturing specialist. He was a member of the Quartermaster Corps of the National Army from September, 1918, until December of the same year. In 1919, he resigned from the Department of Agriculture and became superintendent of a dairy plant in Seattle, Washington. Mr. Trimble returned to the Department in 1922, and remained there until his death. During these years, he investigated problems in buttermaking and creamery management, and supervised the manufacture of sweet-cream butter for the United States Navy. Concurrently he was in charge of the inspection of renovated butter factories. In addition to these duties, he introduced to commercial creameries the Bureau of Dairy Industry's method for manufacturing concentrated cultured buttermilk and the grain-curd method for manufacturing casein. After 1936, his primary duty was to administer the Federal regulatory acts relating to the enforcement of regulations governing the manufacture of process butter, and the inspection and certification of dairy products for export. He aided materially in the preparation of these Federal acts. He also cooperated quite closely with officials of the Bureau of Internal Revenue in their search for violations of Internal Revenue Laws relating to adulterated butter. Mr. Trimble was a prolific writer and speaker and

is the author of a complete but unpublished treatise pertaining to the history of the development of the factory system for manufacturing butter. He was a member of the Masons, the American Dairy Science Association and the Association of the Food and Drug Officials of the United States. He was a member of the Wallace Memorial United Presbyterian Church, in Washington since 1923 and was a trustee in this church for 18 yr and an elder for 3 yr. He is survived by his wife, Mrs. Mary Berry Trimble and one daughter, Mary Patricia Trimble, who reside at 1443 Holly Street, N.W., Washington, D.C.

*Dr. Oliver Ralph Overman*, Professor of Dairy Chemistry at the University of Illinois, passed away Wednesday afternoon, November 23, 1949, at McKinley Hospital in Urbana. He was born in Windfall, Indiana, on April 15, 1886. Dr. Overman came to the University of Illinois in 1917 as Associate Dairy Chemist in the Department of Dairy Husbandry and, from his researches there, became known as one of the outstanding authorities in the United States on the chemical composition of milk. He received the baccalaureate degree from the University of Indiana in 1910, the Master of Science degree from that same institution in 1911, and the Doctor of Philosophy degree in Chemistry from Cornell University in 1917. Before coming to the University of Illinois, Dr. Overman taught at Indiana University, at Cornell University and was Professor of Chemistry and of Geology at Huron College, Huron, South Dakota. Professor Overman was head of the division of Dairy Chemistry in the Department of Dairy Science at the University, having been made an Assistant Professor in 1919, Associate Professor in 1935 and Professor in 1939. His principal scientific interests have been in the electrodeposition of lead, the oxidation of hydrazine and ammonia, and, especially, the chemical composition of dairy products, in which field he was a pioneer and in which he has made notable contributions to science and to the dairy industry. At the time of his death, Dr. Overman was just completing an extensive experiment in which he had been engaged for nearly 3 yr. It dealt with the effects of geographic distribution, season, climate and soil conditions upon the composition of milk produced by Brown Swiss cattle. Dr. Overman had been granted a sabbatical leave by the University for the second semester to study the various conditions in the United States where the milk was being produced and to complete his investigations. Perhaps the work for which Dr. Overman was best known was his detailed study of the energy value of milk, which information is included in most standard works throughout the world on the subject of the nutritive value of milk. He published the results of his extensive

researches in many American, German, and French scientific journals, and was the author of a number of bulletins of the Illinois Agricultural Experiment Station. Dr. Overman was a member of a number of learned societies and scientific groups, including Sigma Xi, Gamma Sigma Delta, Alpha Chi Sigma, the American Dairy Science Association, and the American Chemical Society. He was a member of the Exchange Club of Urbana and had been active in Boy Scout work. He is survived by his wife, Mrs. Olive Spencer Overman of 610 W. Nevada St., Urbana, and two sons, Dr. Ralph S. Overman, a chemist in the Medical College of Cornell University, and Dr. Joseph D. Overman, also a chemist, employed by the DuPont Co. in Parlin, N. J.

*Dr. Arthur Henry Kuhlman*, Professor of Dairying at Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma, died at Stillwater on September 26, 1949. He was born January 1, 1886, at Lowell, Wis., a son of Fred and Anna Kuhlman. Dr. Kuhlman graduated from Juneau, Wis., High School in 1906. He received his B.S. degree in Animal Husbandry from the University of Wisconsin in June, 1910, and his Master's degree from the same institution in 1916. During the period of 1910-14 he was employed as part-time assistant in the Department of Animal Husbandry at the University of Wisconsin. He also held a temporary position as an instructor in Agriculture at Bemidji, Minn. From 1914-17 he was employed as Instructor in Animal Husbandry at the University of Wisconsin. During the year 1917-18 he acted as Emergency Demonstration Agent at Juneau, Wis. From 1918-26 he held the position of Associate Professor of Animal Husbandry at South Dakota State College, Brookings, S. D. He then returned to the University of Wisconsin for further graduate study and was granted the degree of Doctor of Philosophy in genetics in June, 1928. On January 1, 1929, he accepted a position with the Oklahoma Agricultural and Mechanical College as Associate Professor of Dairying. On July 1, 1929, he was raised to the rank of Professor in Dairying at Oklahoma Agricultural and Mechanical College, which position he held until the time of his death. From 1937-1939 he was Acting Head of the Department of Dairying at Oklahoma Agricultural and Mechanical College. Dr. Kuhlman is the author of over 60 scientific publications in the form of bulletins and journal articles. These include several on genetics, but his work dealt very largely with the nutrition of the various classes of livestock, particularly dairy cattle. He did a considerable amount of work on the nutritional value of cottonseed meal, mungbeans and mungbean, peanut and alfalfa hays, as well as several other feeds. More recently his research was confined to the

carotene requirements for growth and reproduction with different breeds of dairy cattle. His work also included several publications on the influence of various feeds on the chemical composition and physical characteristics of butterfat. At the time of his death, Dr. Kuhlman was a member of Alpha Zeta, Sigma Xi, Phi Sigma, Farmhouse, American Association for the Advancement of Science, American Society of Animal Production and American Dairy Science Association. Dr. Kuhlman also was very outstanding in Masonic work, being a member of Frontier Lodge #48 A. F. and A. M., Royal Arch Masons, Knights Templar, White Shrine, Eastern Star and Knights of the York Rites Cross of Honor. This last named order is conferred on only those members of the York Rites who have served as head of all York Rite Masonic bodies. Dr. Kuhlman made two trips abroad where he attended livestock shows and visited livestock farms in England, Scotland, Ireland, the Channel Islands, Holland, Belgium, Germany, Switzerland and France. Dr. Kuhlman was a man who was honored and respected by all who knew him. He was a true gentleman in every respect and played an important part in the life of his community.

*Mark H. Keeney*, Dairy Superintendent at Essex County Hospital, Cedar Grove, for 26 yrs., died October 9, 1949. Mr. Keeney was born in Denver, February 2, 1894. While a child he was taken to Laceyville, Penn., where he attended public schools. He was graduated from Pennsylvania State College, where he majored in dairying. He later received an M.A. from University of Missouri, which he attended on a scholarship awarded for judging cattle at the Chicago International Stock Show. When the United States entered World War I, Mr. Keeney was agricultural agent for Clinton County, Penn. He quit that post to join the Army. After the war he went to University of Missouri as a "dairy specialist." His health soon broke and he retired to spend a year farming in Ohio. In 1921 he came to Rutgers University as chief of dairy extension work, a post he held until going to handle the Essex herd. In the Spring of 1923 the Essex freeholders were considering disposing of the Overbrook Hospital dairy herd because of low milk yield and high cost of production. They decided first to apply to New Jersey College of Agriculture at Rutgers for an expert to make a survey. Mr. Keeney was sent. Within a few years the herd was setting world's records for milk and butterfat production and providing the hospital excellent milk at low cost. The production records soon brought unprecedented demands from dairymen for calves from the Overbrook

herd. Many calves sold to owners of herds in Cuba, Puerto Rico and Central and South American countries. Mr. Keeney's reputation became international, particularly after publication of his *Cowphilosophy*, a handbook on how to develop highly productive dairy herds. The book, which is used as a textbook in numerous agricultural colleges, was a sequel to his *Bullphilosophy*, a short treatise on the breeding of bulls. In 1944 the Holstein-Friesian Association of America announced Mr. Keeney's herd had established a 10-yr. world's record for butterfat and milk production with herds of more than 70 cows. The Essex County Board of Agriculture in 1940 made Mr. Keeney an honorary life member. He had been a member since 1923 and served many years as its secretary. Mr. Keeney was a member of a number of societies and organizations, including Gamma Alpha, Lambda Gamma Delta, Acacia, Kiwanis Club, American Legion, American Farm Bureau, State Board of Agriculture of Pennsylvania and an honorary member of the Voorhees Society of Rutgers University. In 1941 the State Board of Agriculture presented Mr. Keeney its Distinguished Award. He was the youngest man ever to receive that honor. He is survived by his wife, Mrs. Eleanor McCullough Keeney; three sons Mark Keeney, Jr., a graduate student at Pennsylvania State College, where he will receive a doctorate of philosophy; David of Columbus, Ohio, and Philip of Winthrop, Minn., and two grandchildren.

*S. S. Smith*, Director of the Dairy and Food Division, Virginia Department of Agriculture, Richmond, died in December, 1949. He was graduated from Berry School, Mt. Berry, Ga. He operated an ice cream plant at Bristol, Va. and for a period of time he was instructor in dairy short courses at the Virginia Polytechnic Institute. In 1926 he joined the staff of the Virginia Dairy and Food Division as chief dairy inspector and a short time later he was made director of this division. In the loss of Mr. S. S. Smith, the dairy industry of Virginia lost a staunch friend and an untiring worker. His work in improving the standards of quality of the dairy products in the state stands as a monument to him.

Respectfully submitted—H. A. BENDIKEN; C. R. GEARHEART; W. H. E. REID, *Chairman*.

Upon motion duly seconded, the report was accepted.

#### REGISTRATION COMMITTEE REPORT

Frank V. Kosikowsky, Cornell University, made the following report for the Registration Committee:

Number of Persons Registering at A.D.S.A. Meetings, Cornell University, Ithaca, June, 1950.

<i>State</i>	<i>Total</i>
Alabama .....	9
Alaska .....	1
Arizona .....	2
Arkansas .....	2
California .....	8
Colorado .....	3
Connecticut .....	24
Delaware .....	4
D. C. ....	38
Florida .....	28
Georgia .....	12
Illinois .....	136
Indiana .....	21
Iowa .....	27
Kansas .....	11
Kentucky .....	32
Louisiana .....	4
Maine .....	5
Maryland .....	56
Massachusetts .....	36
Michigan .....	56
Minnesota .....	58
Mississippi .....	1
Missouri .....	17
Montana .....	3
Nebraska .....	7
New Hampshire .....	31
New Jersey .....	37
New York .....	346
No. Carolina .....	38
No. Dakota .....	2
Ohio .....	115
Oklahoma .....	3
Oregon .....	5
Pennsylvania .....	87
Rhode Island .....	11
So. Carolina .....	8
So. Dakota .....	4
Tennessee .....	10
Texas .....	6
Utah .....	5
Vermont .....	15
Virginia .....	45
Washington .....	8
West Virginia .....	17
Wisconsin .....	96
Wyoming .....	1
Total .....	1491

Other Countries

<i>Country</i>	<i>Total</i>
Alaska .....	1
Canada .....	27
Costa Rica .....	1
Denmark .....	4
England .....	1
Holland .....	3
Israel .....	1
Peru .....	1

Total .....	38
Grand total .....	1529
Men .....	940
Women .....	394
Children .....	195
Total .....	1529

MEETING OF THE EXECUTIVE BOARD

The Executive Board transacted the following business:

Approved the minutes of the 1949 annual meeting.

Approved the Editor's Report.

Approved the Secretary's Report.

Approved the Journal Management Committee Report.

Approved the Auditing Committee Report.

Approved a Budget for 1951 amounting to \$40,000.

Approved the Resolutions Committee Report.

Elected W. V. Price as a member of the Journal Management Committee to serve for three years (1951-52-53).

Re-employed F. E. Nelson as Editor for the ensuing year (1951).

Re-employed P. R. Ellsworth as Secretary-Treasurer for the ensuing year (1951).

Approved the Honors Committee selection of Martin J. Prucha as Honorary Member.

Voted to recommend to the Association that dues be changed as follows effective January 1951:

Members from \$6.00 to \$8.00

Student affiliates from \$3.00 to \$5.00

Subscribers—no change

Voted to recommend that the membership list be published in the December issue of the Journal every other year, with names of new members only being published on the alternate years.

Voted to recommend that the Association discontinue publication of Student Affiliate membership lists.

Renewed Student Affiliate Branch Certificates for University of Missouri, University of Nebraska, Pennsylvania State College, Clemson, Texas Technological College and State College of Washington.

Appointed W. E. Krauss as American Dairy Science Association representative on the National Research Council.

Recommends that a plan of rotation of annual meeting locations be instituted as a general guide for future meetings whereby meetings may be held in the Midwest every other year (odd) and in the West, South and East in rotation on the alternate years (even). This plan of rotation to be followed only to the extent possible, based on invitations received and other matters beyond the

control of the Association. Final decision to be made by the Executive Board.

Appointed K. L. Turk to succeed himself as American Dairy Science Association representative on the Ralston Purina Co. Research Fellowship Awards Committee.

Voted to recommend approval of the Breed Relations Committee Report.

Adopted Extension Committee Report on Extension Award.

Voted to commend the Milk Industry Foundation for their vision in establishing awards recognizing leadership among Dairy Students in United States and Canada.

The American Dairy Science Association Nominating Committee nominated the following candidates in April: Vice-President, H. A. Bendixen and G. H. Wilster; Directors, J. H. Erb, B. E. Horrall, L. A. Moore, W. H. Riddell.

Results of the election were announced on June 1 as follows: Vice-President, H. A. Bendixen of Washington; Directors, J. H. Erb of Ohio and L. A. Moore of Washington, D.C.

Upon motion duly seconded, the minutes were approved.

President Trout called on R. J. Ramsey who told of plans for the dairy products judging contest at Atlantic City in October, and A. C. Dahlberg who told of the work of the National Research Council on milk regulation studies.

W. E. Petersen moved and F. J. Arnold seconded that all action of the Executive Board during the past year be approved.

## THE AMERICAN DAIRY SCIENCE ASSOCIATION AWARDS

*Ithaca, New York, June 22, 1950*

J. M. Sherman, acting as toastmaster at the annual Awards Banquet at Statler Inn, Cornell University, Ithaca, New York, presented G. M. Trout, President of the Association, who installed the officers-elect as follows: R. B. Becker of Florida as President; H. A. Bendixen of Washington as Vice-President; J. H. Erb of Ohio and L. A. Moore of Washington, D.C. as Directors.

"Mr. Becker, you are about to take over the responsibilities of President of the American Dairy Science Association. As President it will be your duty to be chairman of the Executive Board and submit to the Board for approval the nominations of members to fill vacancies that may occur among the elected officers of the Association. As President you shall appoint, without the approval of the Executive Board, the standing non-elective committees of the Association. With these obligations, privileges and responsibilities, I now charge you with the honor of being President of

the American Dairy Science Association with all the privileges, responsibilities and obligations pertaining thereto."

"Mr. Bendixen, you are about to take over the responsibilities of Vice-President of the American Dairy Science Association. As Vice-President, it will be your duty to preside over the Executive Board in the absence of the President and assume other duties of the Executive Board. At the expiration of President Becker's term, you will automatically become President of this Association. I now charge you with these duties."

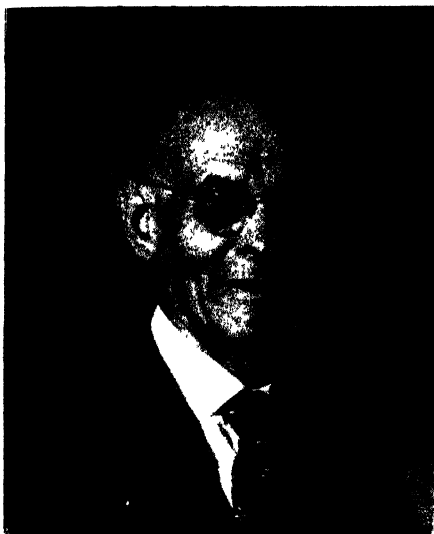
"Mr. Erb and Mr. Moore, you were elected to the Executive Board of the American Dairy Science Association. It is the duty of the Board members to pass on all applications for the establishment of divisions, sections and student branches of the Association. You will have full control of the budget and general business of the Association and have title to all property and funds of the Association. You will be members of the Board that has all the rights and power vested in the by-laws of the Association. With these privileges, responsibilities, and obligations you are now considered as members of the Executive Board of the American Dairy Science Association to serve a term of 3 years."

President Trout then introduced Fordyce Ely, Chairman of the Honors Committee, who read the following citation:

"There comes a time in the life of a man when his friends, associates and colleagues wish to do him honor. Usually it is for a job well done and in such a way as to excite a tribute from fellow workers in the same or closely allied fields of endeavor. Will Martin John Prucha, Professor Emeritus at the University of Illinois, please come forward.

Martin John Prucha was born November 11, 1874, in Jezov, Bohemia. He came to this country at the tender age of 15 yr. to seek his fortune. Having served an apprenticeship as a baker's helper in Bohemia, he began his career in this country working in a bakery in Cleveland, Ohio. Four years on this job convinced this ambitious young man that his future in this country depended on his further education. Accordingly, he enrolled in a Lutheran College (Calvin) for 1 yr. followed by 3.5 yr. at Mt. Herman School for Boys, Mt. Herman, Mass.

He then entered Wesleyan University, Middletown, Conn., graduating in 1903. There he studied under H. W. Conn, often referred to as the father of dairy bacteriology in this country. Next, he accepted a position as Assistant Bacteriologist at the New York Agricultural Experiment Station at Geneva, N. Y., where he worked under the supervision of Dr. H. A. Harding. He earned



MARTIN JOHN PRUCHA

his M.S. degree at Wesleyan University in 1908, studying under Conn and Atwater, the latter a chemist. He accepted a fellowship here at Cornell, 1910-1912, following which he was appointed as an instructor in plant physiology. He was made an assistant professor a short time after completing his work for the Ph.D. degree, studying in the field of physiology of bacteria. A few months later, still in 1913, he accepted an appointment as Assistant Professor of Dairy Bacteriology at the University of Illinois, where he served so well until his retirement in 1943.

In 1907 he married a beautiful young lady, Elizabeth Catchpole, of Geneva, N. Y. Three children blessed this union: A. A. Prucha, County Sanitary Civil Engineer, Marin County, Calif.; Mrs. R. C. (Marjorie) Hodgman, 703 Arlington Road, Penn Valley, Penn.; and M. J. Prucha, Jr., Geo-physicist for the Shell Oil Co., Houston, Texas.

Dr. Prucha's research has been largely in the field of dairy farm and dairy plant sanitation. His earliest work had to do with a chemical study of the ripening of cheese. His exhaustive studies of the sources of bacteria that gain entrance to milk and dairy products are classic. From his extensive studies of the effect of different gases upon bacteria, has evolved the process of whipping cream by charging it with gas followed by a quick release of the pressure. His work dealing with the sanitary aspects of paper milk containers led to their adoption by the Chicago Health Department and later by other health departments

throughout the United States. Dr. Prucha was one of the early workers in the field of chemical sterilizers. He devoted much of his time to research to determine the relationship of chemical sterilizers and washing powders to the corrosion of metals. Within the past year Dr. Prucha was presented with an award by the Chicago Dairy Technology Society in recognition of his outstanding career as a dairy scientist.

Dr. Prucha has been a good servant of the dairy industry. He is loved by his students and admired and respected by his fellow workers. He is cheerful at all times and enthusiastic about the future of the dairy industry. He has always championed the cause of high quality milk and dairy products, and he has emphasized character and proficiency in his work with students. His philosophy has been, "Do what you think is right and don't worry about it."

Dr. Prucha is also an active worker in the Episcopal Church and a member of the Exchange Club. His undergraduate social fraternity was Delta Tau Delta. He is a member of Gamma Sigma Delta honor society and Sigma Xi. He has been a member of the American Dairy Science Association for many years and a liberal contributor to the *Journal of Dairy Science*. He is also a member of the American Public Health Association and the International Association of Food and Milk Sanitarians.

Though retired for 7 yr., Dr. Prucha is still active. He continues to carry on a limited amount of research work and regularly attends industry and professional meetings. He has a summer home at Christian Assembly, Frankfort, Mich., and his legal residence is 702 W. Nevada, Urbana, Ill.

It seems singularly appropriate to honor Dr. Prucha while our meeting is here at Cornell, where he spent several happy years.

Dr. Prucha, by virtue of authority vested in me as chairman of the Honors Committee by our Executive Board, it is indeed a pleasure to confer upon you an honorary membership in the American Dairy Science Association, a small tribute to your years of service to the dairy industry as a dairy scientist, a teacher and a friend to all."

I. A. Gould, Chairman of the Borden Award Committee for Manufacturing, was introduced and read the following:

"The man selected for the 1950 Borden Award in Dairy Manufacturing is recognized as a researcher, a teacher and a counselor of men. He has left his imprint in the advancement of dairy science not only by the research he has conducted or directed, but also by the training and assistance he has given others so that they, in turn, would be equipped and inspired to continue to make further contributions to the dairy industry.



This year's recipient has been engaged in research and teaching for 25 yr. During this span, he has been author or co-author of almost one hundred scientific and popular papers, reports and bulletins. More than one-third of the publications are original scientific papers, the majority of which have been published in the *Journal of Dairy Science*. He has given unstintingly of his time as a major professor, teacher, adviser or counselor for a large number of students conducting work for advanced degrees, many of whom have since assumed key positions in the dairy industry. In addition, during the past 20 yr. he has instructed more than 1,000 students in short-course and 4-yr. programs.

His research has been almost entirely confined to the field of cheese, and his selection for the award is based primarily on fundamental and applied research dealing with cheddar and brick varieties. He early demonstrated that pasteurized milk could be used successfully for cheesemaking and established proper processing and manufacturing procedures for pasteurized milk cheese. Through a series of researches, he revealed the necessity of maintaining proper pH and acidity control during the cheesemaking operation in order to insure the cheese against the development of objectionable flavors during storage. Within recent years, he has been associated with fundamental research dealing with the enzyme system of cheddar cheese, particularly with the lipase and proteinase enzymes and the chemical changes which result from their action. Another series of studies were made which revealed the relationship of the diacetyl content of cheddar cheese to flavor production. Research was also conducted and publication made on the development and application of methods for testing cheese for fat and moisture and for analyzing cheese for extraneous material. His studies on these analytical procedures serve as a basis for methods and techniques now generally used by the cheese industry. In addition, he has given attention to problems of cottage and cream cheese, to the relationship of calcium chloride concentration to rennet action, and to the mechanization of cheesemaking practices.

The choice for the 1950 award was born in Schenectady, N. Y., in 1896. He served in the United States Navy during World War I. He attended Cornell University both as an undergraduate and a graduate, receiving the Bachelor of Science Degree in 1920, the Master of Science in 1921 and the Doctor of Philosophy in 1925. Prior to the completion of his formal education, he gained commercial experience by working in market milk and ice cream plants in New York State. He served on the staff at Cornell University for about 4 yr. following receipt of his doc-

tor's degree and then accepted a position as Professor of Dairy Industry at the University of Wisconsin in 1929—a position he still holds.

He has served as a director of the American Dairy Science Association and as a member of committees of the Association concerned with cheese research and standards. He is the co-author of a textbook on cheesemaking which is accepted by the Industry as a standard reference book.



WALTER VAN PRICE

On the basis of his outstanding contributions to our knowledge of the technology of cheese manufacturing, with particular reference to the cheddar and brick varieties, the Borden Award Committee for Dairy Manufacturing of the American Dairy Science Association has chosen Dr. Walter Van Price, Professor of Dairy Industry, University of Wisconsin, for the 1950 award."

Mr. W. A. Wentworth of the Borden Company Foundation presented Dr. Price with a gold medal and check for \$1,000.00.

G. W. Salisbury, Chairman of the Borden Award Committee for Production, then was introduced and made the following statement:

"Research in the field of dairy production has undergone profound changes since studies in this field were first introduced into the routine curricula of American colleges nearly a half century ago. Especially during the last 25 yr. have these

changes been directed more and more towards emphasis on the fundamentals of the vital chemistry and physiology of the dairy cow and the lactation function. The 1950 recipient of the Borden Award in Dairy Production has witnessed many of these changes and during the past 25 yr. has contributed as much as any living man to knowledge of the fundamental energy metabolism of the dairy cow. A naturalized American citizen, he was born on a general farm in Garbatchi, Lithuania, on February 8, 1890. He came to the



SAMUEL BRODY

United States as a young man and received his American citizenship in 1912. He received the A.B. degree from the University of California in 1917, was for a time in the Department of Chemistry and Physics in the College of Physicians and Surgeons of San Francisco, and then entered the United States Army, first in aviation and later in chemical warfare service, 1917-1918. He was Assistant in Biochemistry at the University of California from 1919 to 1920, when he received the M.A. degree.

He joined the Department of Dairy Husbandry at the University of Missouri in 1920 as an Assistant Professor and has risen through the successive ranks to a professorship in that Department. He received the Doctor of Philosophy degree from the University of Chicago in 1928, his work having been supported in part by grants from the National Research Council. Later, he

was awarded a Guggenheim Memorial Foundation Scholarship and studied at Strasbourg, Paris, Berlin, Copenhagen and Moscow during 1930-1931. He served as chairman of the joint committee between Agriculture and Biological Chemistry for the Herman Frasch Foundation Studies at the University of Missouri from 1929 to 1940. These studies led to the production of a large number of papers on the general subject of growth and development with special reference to domestic animals. In these studies, a wide area of subject matter was covered and many contributions to knowledge made. The major emphasis was on dairy cattle and from this work our recipient published 65 research bulletins of the Missouri Agricultural Experiment Station. From 1921 to date, he has contributed 62 articles to scientific journals, and has written a number of scientific reviews on his specialty, including the section on growth for the recent edition of the Encyclopedia Britannica.

Dr. Samuel Brody, the 1950 recipient of the Borden Award in Dairy Production, is author of the monumental work *Bioenergetics and Growth*. During the period 1944 to 1949, he has published 14 research bulletins of the Missouri Agricultural Experiment Station, most of them dealing with the relationship of temperature to the energy metabolism of cattle and the milking function. He has published 15 scientific articles in research journals, most of which deal with the effects of growth and aging upon domestic animals. It is on the basis of these studies that the Dairy Production Committee unanimously selected Dr. Brody as the 1950 recipient of the Borden Award in Dairy Production. From his work, many of the dairy cattle management practices of the future will rest on a firm, scientific basis.

On behalf of the Committee on the Borden Award for Dairy Production and our colleagues in the Production Section of the American Dairy Science Association, it is a pleasure to present Dr. Samuel Brody, Professor of Dairy Husbandry at the University of Missouri, to receive the Award."

Mr. W. A. Wentworth of the Borden Company Foundation presented Dr. Brody with a gold medal and a check for \$1,000.00.

#### AMERICAN FEED MANUFACTURERS' AWARD

R. B. Becker, Chairman of the American Feed Manufacturers' Award Committee, was introduced and spoke as follows:

"For 3 yr., the American Feed Manufacturers' Association has encouraged superior original research in dairy cattle nutrition, and recognized the worker who made the outstanding contribu-

tions, under rules established by the American Dairy Science Association.

The Committee for the American Feed Manufacturers' Association Award received 14 nomina-

tions this year, and found 12 others eligible from work published in dairy nutrition during 1948 to 1949. Evaluation of the publications narrowed the list to eight close contenders. From these the candidate was named on the first ballot.

The research which made the candidate eligible appeared in four technical journals during the prescribed period. It added to knowledge concerning bovine saliva, an indicator method for digestibility of forage by ruminants, efficiency of simple rations for dairy bulls, calcium and manganese metabolism with cows and calves. A number of collaborators assisted the author with the investigations.

The candidate was schooled in Maryland and Michigan. He has been engaged in dairy nutrition research for 10 yr. at Michigan, New Jersey and New York, and is one of our hosts at this delightful annual meeting. He is Dr. J. Thomas Reid.

Please come forward, Dr. Reid.

Dr. H. Ernest Bechtel, chairman of the Nutrition Council of the American Feed Manufacturers' Association, will present the award.

Dr. Bechtel, our Committee presents as candidate of the American Dairy Science Association for the American Feed Manufacturers' Association award—Dr. J. Thomas Reid."

H. E. Bechtel, Chairman of the Nutrition Council of the American Feed Manufacturers' Association, then presented Dr. Reid with a check for \$1,000.00.



J. THOMAS REID

# JOURNAL OF DAIRY SCIENCE

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## ALDOLASE IN BOVINE MILK

B. D. POLIS AND H. W. SHMUKLER

*Eastern Regional Research Laboratory,<sup>1</sup> Philadelphia 18, Pennsylvania*

The enzyme aldolase which reversibly splits fructose 1,6-diphosphate into dihydroxyacetone phosphate and phosphoglyceric aldehyde was first discovered by Meyerhof and Lohmann (3) in rabbit voluntary muscle. The enzyme probably occurs in all cells, but muscle and yeast are the best sources. More recently, the enzyme has attracted attention because the experiments of Warburg and Christian (6) indicated an increase of aldolase in the serum of tumor-bearing rats. With the known relationship of the serum and milk whey proteins (2) in mind, it was of interest to determine the possible presence of aldolase in normal milk.

### EXPERIMENTAL

The procedure described in detail by Sibley and Lehninger (5) was applied directly to milk without any modifications. The method depends on the formation of a 2,4-dinitrophenylhydrazine derivative of the triose phosphate produced by the action of aldolase on hexosediphosphate. In alkaline solutions, the dinitrophenylhydrazine derivative, called chromogen, turns purple. The assay of whole or skim milk for aldolase activity was complicated by turbidity in the solution of the chromogen after addition of NaOH. This occurred with normal milk or milk inactivated by trichloroacetic acid. The turbidity did not appear with milk that had been dialyzed or with milk aldolase preparations made by salt fractionation. To eliminate the turbidity, the colored solution was centrifuged immediately before it was compared with acid-inactivated milk treated in a similar manner. The aldolase activity of milk determined by this procedure was identical with that of a dialyzed sample of the same milk. No further difficulty was encountered with the aldolase assay.

Aldolase activity may be conveniently expressed as the micromoles of 1,6-fructose diphosphate split by 1 mg. of protein at 37° C. in 1 hr. One micromole of hexosediphosphate is equivalent to 2 micromoles of triosephosphate or 2 micromoles of alkali-labile phosphate. The equivalence between the triose chromogen and alkali-labile triosephosphate was determined with a rat-muscle preparation of aldolase. The aldolase activity, stated in terms of micromoles of hexosediphosphate split, multiplied by the factor 22.4 is equivalent to the  $Q_{\text{HDP}}$  used by Sibley and Lehninger (5). For comparison with their data, this  $Q$  notation is used.

Received for publication February 4, 1950.

<sup>1</sup> One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. D. A.

Protein concentrations were determined by the biuret color reaction described by Kingsley (1). Milk was analyzed within a few hours after collection or after storage overnight at 3° C. in the presence of chloroform. Both mixed commercial skim milk from a Philadelphia dairy and whole milk from each of six animals were analyzed. The results are summarized in table 1.

TABLE 1  
*Aldolase activity of normal milk and various milk fractions*

	$Q_{HDP}$	
	Range	Average
Whole milk <sup>a</sup>	0.07-0.13	0.09
Cream <sup>b</sup>	0.14-0.45	0.34
Skim milk <sup>c</sup>	0.07-0.22	0.13
Whey (casein precipitated with acid)		0.00
Whey (casein coagulated with rennet)	0.15-0.35	0.22
<i>Rennet whey salt fractionation at 26° C.</i>		
2.3 M ammonium sulfate ppt. <sup>d</sup>		0.41
2.8 M " "		0.00
<i>Salt fractionation at 3° C.</i>		
2.4 M ammonium sulfate ppt. <sup>d</sup>		2.3
2.8 M " "		6.8

<sup>a</sup> Whole milk from each of four cows was analyzed.

<sup>b</sup> Whole milk was centrifuged for 10 min. at room temperature (3000 rpm), and the skim-milk was siphoned off. One ml. from the center of the recentrifuged cream layer was used for the aldolase determination.

<sup>c</sup> From two 15-gal. lots of mixed unpasteurized commercial skim milk and eight lots of milk from six cows.

<sup>d</sup> The fractions were dialyzed free of salt before enzyme assay. The pH was then  $6.4 \pm 0.1$ .

<sup>e</sup> Casein was precipitated at 1.5 M  $(\text{NH}_4)_2\text{SO}_4$  concentration.

## RESULTS AND DISCUSSION

The activity of milk aldolase is of the same order as that reported for blood serum (5). Like xanthine oxidase, this milk enzyme is concentrated in the cream layer. Use of a conventional salt fractionation procedure at room temperature was complicated by the instability of the enzyme in milk. Removal of casein by isoelectric precipitation at pH 4.7 resulted in a complete acid inactivation of the whey aldolase. Although there was an apparent increase of the  $Q_{HDP}$  value when the casein was removed with rennet and the enzyme was precipitated at a concentration of 2.3 molar  $(\text{NH}_4)_2\text{SO}_4$  at room temperature, there was a loss of almost two-thirds of the total enzyme activity. The removal of casein with 1.5 M  $(\text{NH}_4)_2\text{SO}_4$  and the subsequent fractionation of the whey at 3° C. concentrated the milk aldolase in the fractions that precipitated at concentrations of 2.4 and 2.8 molar salt. Approximately 80 per cent of the total activity in milk could be recovered with this procedure. In the fraction that precipitated at a concentration of 2.8 molar  $(\text{NH}_4)_2\text{SO}_4$  there was about a fifty-fold increase in purity ( $Q_{HDP} = 6.8$ ).

Some explanation for the loss of activity with the rennet whey was obtained in the subsequent study of the effect of temperature on the stability of the milk aldolase. Figure 1 demonstrates a marked instability of the enzyme in milk at 37° C., as compared with the stability of the relatively purified milk aldolase. The

linear relationship for the plot of the log per cent aldolase activity remaining after heating against the time of heating reveals a simple monomolecular inactivation rate for the enzyme in milk at 37° C. A similar curve was obtained with dialyzed milk, indicating that the instability of the aldolase in milk could not be attributed to dialyzable components. Although the purified aldolase fraction showed no inactivation at 37° C., at 48° C. the activity of this fraction diminished rapidly but still followed a monomolecular reaction rate. The complicated inactivation rate of the aldolase in milk at this temperature probably indicated inactivation due to heat and unknown degradative changes.

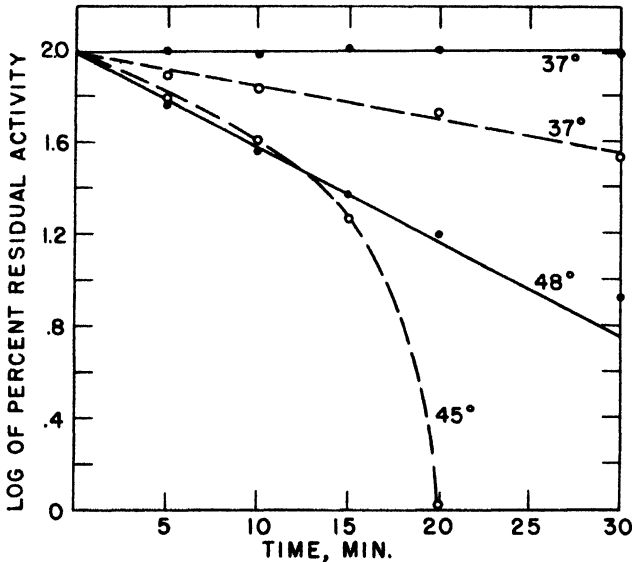


FIG. 1. Aldolase activity of normal milk (○) and a purified milk fraction (●) after heating at various temperatures for progressive time intervals. The pH was 6.4 in both cases. The purified fraction had been dialyzed free of salt.

This rapid inactivation of the aldolase in milk, in the light of the relative stability of the aldolase in fractions from milk, permits the conclusion that milk contains non-dialyzable components capable of destroying or inactivating the aldolase. Similar factors have been found in the crude extracts of muscle aldolase.

The presence of aldolase in milk with an activity level close to that reported for blood serum ( $Q_{\text{HDP}} = 0.3$ ) emphasizes further the close relationship of the proteins of serum and milk whey (2). In view of the reported presence of xanthine oxidase in both bovine blood serum and milk and its absence in both human serum and milk (4), the presence of aldolase in both serum and milk constitutes further presumptive evidence of the possible origin of certain milk enzymes.

## SUMMARY

With the procedure of Sibley and Lehninger, the enzyme aldolase has been found in normal cow's milk in the same concentration range as in blood serum. The presence of the enzyme in various milk fractions is indicated, and factors affecting the stability of the enzyme in milk are discussed.

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# INFLUENCE OF PRE-MILKING PREPARATIONS OF COWS' UDDERS UPON THE LET-DOWN OF MILK

C. E. KNOOP AND C. F. MONROE

*Department of Dairy Industry, Ohio Agricultural Experiment Station, Wooster*

Improvements in the machine milking of cows have been attained through practical experiences and from findings obtained in experimental work (1, 9, 10, 13, 20). Studies (5, 17) on the anatomy of teats and udders, together with those (14, 15) on the effect of the amount of vacuum at the inflations have contributed valuable lessons regarding mechanical milking. Along with the mechanical features in machine milking, the importance of good management of the cows themselves should be emphasized, as shown by the work of Whittleston and Verrall (19). Since it has been shown (1, 12) that the improper use of milking machines may result in injuries of the teats and udders which, if continued, may cause inflammation of the udder (10), it is important to know and follow the proper procedures.

In a study of milking procedures, Dahlberg (2) showed that with the right management the milking job could be performed more satisfactorily and as quickly with two units as with four. The importance of preparing the cows for the milking act by the use of the strip cup together with washing the udders has been demonstrated by Miller and Petersen (11), Smith and Petersen (16) and Ward and Smith (18). These workers also showed that the milking machines should be attached soon after the pre-milking preparations are made, preferably within 1 min. When the interval was as long as 20 min. after the treatment, lowered milk production resulted. Knodt *et al.* (6) agreed on the short interval but claimed that with special training a 20-min. interval caused no adverse effect on milk production.

A program of "3-min." milking has been recommended (21); this is based on properly preparing the cow and attaching the machine within 1 or 2 min. Several features of this work have been studied at the Ohio Agricultural Experiment Station by the senior author (7) and reported in 1948. During the progress of this work, which was started in 1946, several reports have appeared on the effect of various factors on the let-down of milk.

Dodd and Foot (3) found that the temperature of the water used in washing cows' udders had no effect on the length of the milking period or total milk production. They (4) have confirmed these findings in a more recent study. In their second experiment, an attempt was made to increase the milking rate by removing the milking machine before the milk flow stopped. This practice not only failed to change the rate of milk flow, but caused a drop in milk production.

Korkman (8) showed that the reaction of the cow to pre-milking udder treatments seems to be an individual characteristic. Milk yield was not influ-

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enced by the various methods of udder treatments. The maximum rate of milk flow per minute was greater when stimulation for the let-down was applied 1 or 2 min. before attaching the teat cups. A light massage with a hot (wet) towel for 15 or 30 sec. was the best stimulation. This management treatment of the udders was most important with low-producing cows.

#### EXPERIMENTAL

The cows used were purebred Holsteins and Jerseys that were in either the fore or middle part of their lactation periods. They represented part of the main dairy herd and, therefore, were accustomed to managed milking procedures. A special effort was made to maintain uniform conditions of feeding and management throughout the work, with the exception of the experimental treatment being used. Each milking trial was for a minimum of 8 days, the

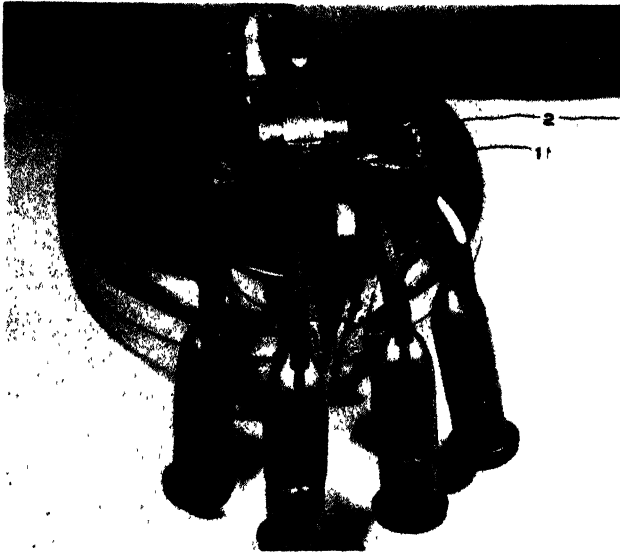


FIG. 1. Experimental milking machine. Wires numbered 1 and 2 are attached to the rotary valve for the control of the first and second latex bag, respectively.

first 4 days of which were considered as preliminary and the second 4 days as experimental. The same procedures were followed for both the morning and evening milkings, but the data presented are for the morning milking, since conditions were more uniform at this time of day.

A Surge milker was used and operated according to the manufacturer's recommendation at 48 to 52 pulsations per minute and with a vacuum of 15 in. of mercury. The pail was so constructed (figures 1, 2 and 3) that the milk obtained in the first and second 45 sec. could be weighed separately. This was made possible by the use of latex bags inside the pail into which the milk was diverted by a valve arrangement on the milking head. After these two 45-sec. periods, the remaining milk was collected in the pail proper. In the first trial,

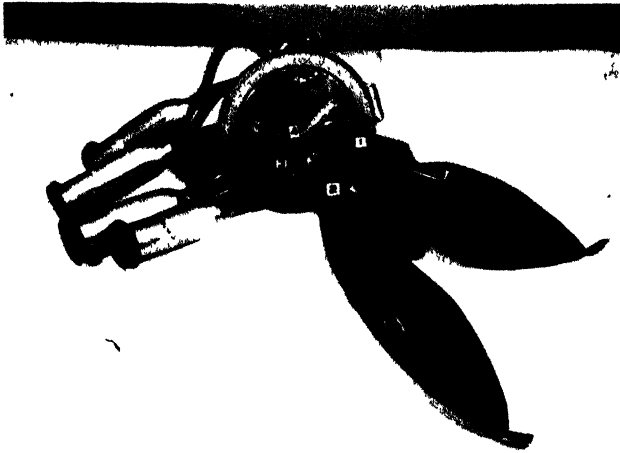


FIG. 2. Lower side of lid and valve with latex bags attached.

only one bag was available so that one measure of let-down was based on the production in the first minute. One man handled the milker and another timed the operations with a stop watch and recorded data.

The time for cleaning or massaging each udder ranged from 10 to 15 sec. A 1-min. period between the preparation of the cow and the attachment of the milking machine was used as standard procedure. The inflations (teat cups) were removed from each teat as soon as the milk flow stopped, and the last one removed determined the length of the milking period.

The response or milking performance of the cows to various pre-milking udder treatments has been measured in three ways. These are explained as follows:



FIG. 3. Pail lid with disc valve and two latex bags.

(a) Pattern of milk let-down. This refers to the percentage (relationship) of the amount of milk removed during the first 45 sec. to that removed during the second 45 sec. of the milking period. The purpose of this measure was to emphasize any production change during the first 1.5 min. of milking. It was reasoned that the speed of let-down and flow of milk of which cows are capable would (in most cases) be established during the second 45 sec. of milking time, regardless of the pre-milking treatment. A high percentage factor indicates that the pre-milking treatment used was an efficient stimulation for speedy let-down. Because it was found preferable to compare cows of similar characteristics as regards ease of milking, the data are presented in this form whenever possible.

(b) One-and-one-half minute production. The pounds of milk obtained during the first 1.5 min. is expressed as a percentage of the total production.

(c) Milking rate. The rate of milk flow equals the milk production (pounds) divided by the milking time (seconds).

The information obtained under *b* and *c* was a further measurement of the efficiency of the pre-milking treatment for the stimulation of speed of let-down, but was somewhat less critical than under *a*.

*Experiment I. The influence of temperature of the udder wash water.* The 16 cows (Holsteins and Jerseys) used were divided into three groups of five, five and six cows. The first group was milked during the summer season, the second during the spring and the last group in the winter season. Each group was subjected to three different temperatures of udder wash water and use of a strip cup (removing one or two streams of milk from each quarter) previous to milking. The rotation of treatments of the udders for groups 1 and 2 were in the following order: cleaning with a heavy Turkish towel wrung from water at 100° F., cleaning as above except the towels were wrung from water at 132° F., and in the last treatment the towels were wrung from cold water. Temperature of the cold water was 64° F. during the summertime and 50° F. during the spring. Each period of treatment was for 8 days. The third group of six cows (table 3) was arranged and experimentally treated according to the "Latin square" system. Under this system they were divided into three groups of two cows each, thus permitting udder applications with 45, 100 and 132° F. waters to be used simultaneously. The first group received cold water treatment followed by the use of warm and then hot water. The second group received, at the same time, a similar series of treatments with warm, hot and cold waters, while the third group received hot, cold and warm water udder treatments.

The influence of temperature of the udder wash water upon the milking response of cows during the summer, spring and winter is presented in data given in tables 1, 2, and 3, respectively. The results have been classified according to the milking characteristics of the cows, because easy-milking cows require less time to be milked than do hard-milking cows.

Temperature of the water used on the udders previous to milking was of minor importance, at least after the cows had become accustomed to a certain temperature change. In six of the eight comparisons, let-down appeared to be

TABLE 1

*Temperature of udder wash water as related to the let-down of milk  
(Summer trial, Experiment I, Trial I)*

Cows	2			2			1		
Milking characteristics	Slow (hard)			Medium			Rapid (easy)		
Temp. udder wash water (° F.)	100	132	64	100	132	64	100	132	64
Milk production, a. m.									
1st. minute (lb.)	5.2	4.9	4.8	6.2	6.2	6.8	7.3	7.7	8.3
1st. minute (% of total)	24.3	22.1	22.4	37.1	38.2	46.6	38.6	41.4	45.9
Total (lb.)	22.2	22.1	21.4	16.7	16.2	14.6 <sup>a</sup>	18.9	18.6	18.1
Time (min. & sec.)	4-29	4-37	4-28	3-17	3-12	2-31	2-42	2-32	2-16
Milking rate (lb./sec.)	0.083	0.080	0.080	0.085	0.084	0.096	0.120	0.120	0.130

<sup>a</sup> Unable to explain this drop. These two cows began to drop in production during later period of hot water treatment, or just before the cold water treatment was started.

just as good using water at 45, 50 or 64° F. as it was with water at 100 or 132° F. Similarly, the response of the cows to warm water (100° F.) was just as good as was the use of hot water (132° F.) in six out of eight comparisons. There were some indications that water at 132° F. was a little too hot for the cows, as shown at times by their stepping around when their udders were being washed and by the lower percentage of the pattern of let-down as shown in the tables. However, one cow in the winter trial seemed to prefer the hot water, as shown by her response (table 3, medium classification). The men doing the milking preferred to use water at 100° F.

The temperature of the udder wash water apparently was without effect on the time required to milk the cows. The time varied slightly with the different temperatures of water used, but there was no definite trend. Use of hot water did not make a fast milker out of a slow one and cold water did not make slow milkers out of fast ones, after the cows had become accustomed to the change in

TABLE 2

*Temperature of udder wash water as related to the let-down of milk  
(Spring trial, Experiment I, Trial II)*

Cows	2			1			2		
Milking characteristics	Slow (hard)			Medium			Rapid (easy)		
Temp. udder wash water (° F.)	100	132	50	100	132	50	100	132	50
Milk production, a. m.									
1st. 45 sec. (lb.)	2.8	2.8	3.0	3.9	3.5	4.1	5.0	4.7	5.3
2nd. 45 sec. (lb.)	3.4	3.6	3.5	4.3	4.2	4.4	5.1	5.5	5.7
Pattern of let-down (%)	82.6	77.7	85.7	90.7	83.3	93.2	98.0	85.4	93.0
1st. 1.5 min., (% of total)	43.4	40.2	41.1	42.2	35.3	40.7	53.7	48.3	53.4
Total (lb.)	14.3 <sup>a</sup>	15.9	15.8	19.4 <sup>a</sup>	21.8	20.9	18.8 <sup>a</sup>	21.1	20.6
Time (min. & sec.)	4-14	4-31	4-24	3-20	3-54	3-40	2-40	2-56	2-36
Milking rate (lb./sec.)	0.056	0.059	0.060	0.097	0.093	0.095	0.120	0.120	0.130

<sup>a</sup> Roughage consumption increased by 4.8 lb. of hay, or its equivalent, per cow per day during the latter part of this period and throughout the experiment.

TABLE 3  
*Temperature of udder wash water as related to the let-down of milk*  
*(Winter trial, Experiment I, Trial III)*

Cows	3			3		
Milking characteristics	Medium			Rapid (easy)		
Temp. udder wash water ( $^{\circ}$ F.)...	100	132	45	100	132	45
Milk production, a. m. ....						
1st. 45 sec. (lb.)	3.9	4.1	3.9	4.9	4.7	5.1
2nd. 45 sec. (lb.)	4.3	4.4	4.3	5.1	5.3	5.3
Pattern of let-down (%)	90.7 <sup>a</sup>	93.2	90.5	96.1	88.6	96.2
1st. 1.5 min. (% of total)	37.1	37.6	37.6	47.1	47.0	47.7
Total (lb.)	22.1	22.6	21.8	21.2	21.3	21.8
Time (min. & sec.)	4-30	4-05	4-26	3-13	3-14	3-08
Milking rate (lb./sec.)	0.082	0.092	0.082	0.110	0.110	0.110

<sup>a</sup> Determined by relating the amount of milk produced during the first 45 sec. to that produced during the second 45 sec. of milking time.

temperatures. Some cows milked completely dry after 2 min., while others required 6.5 min., irrespective of treatment.

*Experiment II. The influence of various pre-milking udder treatments.*

*Trial I.* Five cows (three Holsteins and two Jerseys) in the early and middle stages of lactation were used in this experiment according to the following order of udder treatments (table 4): (a) Control period in which no pre-milking treatment of the udders was given, except placing the surcingle over the back of the cows (see management employed for previous herd practices); (b) dry hand rub (brush) of the udder to remove straw and any loose dirt; (c) cleaning the udder with a damp cloth (half of a Turkish towel) that previously was wrung from water and stored in a dry bucket until used at milking time (these towels were cold when used); (d) cleaning the udders with a heavy towel (half of a Turkish towel) wrung from hot water ( $120^{\circ}$  F.); and (e) the same udder treatment as in (d), followed by removing one or two streams of milk from each

TABLE 4  
*Influence of udder treatment upon the let-down of milk*  
*(5 cows; Experiment II, Trial I)*

Treatment of udders 1 min. before milking	None	Dry hand	Damp cloth	Towel from hot water ( $120^{\circ}$ F.)	Towel from hot water ( $120^{\circ}$ F.) and strip cup
Milking characteristics	1 slow and 4 medium				
Milk production, a. m.					
1st. 45 sec. (lb.)	2.8	3.3	3.8	3.8	3.8
2nd. 45 sec. (lb.)	4.1	4.2	4.2	4.2	4.2
Pattern of let-down (%)	68.3 <sup>a</sup>	78.6	90.5	90.5	90.5
1st. 1.5 min. (% of total)	38.3	41.9	47.3	47.9	49.7
Total (lb.)	18.0	17.9	16.9	16.7	16.1 <sup>b</sup>
Time (min. & sec.)	4-33	4-04	3-30	3-25	3-25
Milking rate (lb./sec.)	0.066	0.073	0.080	0.081	0.079

<sup>a</sup> Determined by relating the amount of milk produced during the first 45 sec. to that produced during the second 45 sec. of milking time.

<sup>b</sup> One cow dropped from an average of 20.1 lb. of milk to an average of 17.6 lb. during this trial. Unable to explain this except stage of lactation.

teat (strip cup). Each of these periods were for 8 days duration with the first 4 days being considered as preliminary and the last 4 days, experimental.

Hot water at 132° F., as used in experiment I, appeared to be too hot for cows' udders and so water of 120° F. was resorted to in this second experiment.

The data obtained in this first trial on pre-milking treatments are shown in table 4. With no treatment (control period), the let-down of milk was relatively slow and the average time required to milk a cow was approximately 1 min. longer than with some of the other treatments. Brushing the udder with the hand was of slight benefit in speeding up the let-down and in shortening the time required to get the milk. The other three treatments—the use of the damp cloth, the towel from hot water and this treatment plus the use of the strip cup—seemed to be about equally effective for the efficient simulation for speedy let-down of milk. Thus, the amount of milk obtained in the first 45 sec. with all three of these treatments was 90.5 per cent of that obtained in the second 45 sec. Likewise, the average milking time following these treatments was reduced by 1 min.

To begin the milking act without pre-milking cleaning or massaging of the udder with a damp or wet towel permits the removal of milk from the cisterns and ducts, resulting in the milking of a dry teat before full milk flow started. According to Petersen (12), this may cause teat injury. Under this method the milking act becomes the stimulus for milk let-down as readily experienced when milking is done by hand.

Cleaning udders with cloths removed from 120° F. water failed to change the amount of milk produced during the first 1.5 min. of milking time, pattern of milk let-down or milking rate when compared with the use of a damp cloth that was previously stored in a dry bucket.

*Trial II.* Completion of trial I revealed that information on the milking response should be obtained upon first, the influence of removing one or two streams of milk from each quarter (strip cup) and, second, a combination treatment of the udders with a bath of hot water (120° F.) and use of a strip cup.

Four cows (three Holsteins and one Jersey) were used in this trial. Pre-milking treatments were used in the following order: (a) no treatment, except placing the surcingle over the cow; (b) use of the strip cup only; (c) massaging the udder with a towel wrung from water at 120° F. and use of a strip cup; and (d) the same as the preceding treatment except that water was allowed to stay in the towel so that this treatment amounted to almost bathing the udder with hot water. Each pre-milking treatment lasted for a period of 8 days, as previously explained.

The cows in this second trial were slower milkers than were those used in the previous trials (table 5). The results agree, in general, with those obtained in trial I. With no treatment previous to milking, the let-down of milk was poor or slow and the milking time was longer than when proper treatments were made. Use of the strip cup slightly improved milk let-down and shortened the milking period. However, desired milking responses were obtained with the use of a damp cloth plus the strip cup. This shortened the milking time by approximately 1 min. and gave a pattern of let-down of 85.7 per cent. The more thor-

ough washing of the udder, which amounted to almost a bath, did not appear to be superior to the use of a wet towel and use of a strip cup; in some instances the cows showed evidences of being disturbed by this more drastic procedure.

As a check upon the milking technique, three special milking experiments were conducted. The influence of increasing the vacuum 20 per cent (to 18 in.) upon the milking response of hard-milking cows was studied. This technical study showed that controlling the vacuum was necessary for satisfactory employment of the procedures used in the experiments.

Since this work was done with a Surge milker, the milking rate conceivably might be slow at the beginning of the milking period because of a lack of weight in the pail. This was investigated and found to be of no consequence.

TABLE 5  
*Influence of udder treatments upon the let-down of milk*  
(4 cows,<sup>a</sup> Experiment II, Trial II)

Treatment of udders 1 min. before milking	None	Strip cup	Towel from hot water (120° F.) and strip cup	Hot water (120° F.) bath and strip cup
Milking characteristics	2 slow and 2 medium			
Milk production, a. m.				
1st. 45 sec. (lb.)	2.7	3.2	3.6	3.4
2nd. 45 sec. (lb.)	3.7	4.2	4.2	3.8
Pattern of let-down (%)	73.0	76.2	85.7	89.5
1st. 1.5 min. (% of total)	28.4	32.3	37.1	32.7
Total (lb.)	22.5	22.9	21.0 <sup>b</sup>	22.0 <sup>c</sup>
Time (min. & sec.)	5-49	5-31	4-55	5-15
Milking rate (lb./sec.)	0.064	0.069	0.071	0.070

<sup>a</sup> Data from a fifth cow was not used because 9.75 to 10.75 min. of milking time was required. All treatments failed to shorten the milking time, except when an increase of vacuum was used.

<sup>b</sup> Warm weather retarded appetites, thereby resulting in lowered milk production.

<sup>c</sup> Appetites improved as a result of cold weather.

The influence of administering additional oxytocin to two cows was studied. Results show that the measurements of the milking responses used (in experimental trials) apparently were sound and that desirable stimulation for speedy let-down of milk had been obtained.

#### DISCUSSION

The milking response was the same with cold, warm or hot water. This is in agreement with the findings obtained by Dodd and Foot (4). This proved to be true for spring, summer and winter. These results were obtained after the cows had become accustomed to a certain temperature of water. There were some indications that the water could be too warm for best results and have a disturbing effect on the cows. In managed milking, great value has been attached to the use of hot water as a means of increasing the milking response or let-down and shortening the total milking time. In this work demonstration of this value for warm or hot water over cold water was not possible.

Various pre-milking treatments of the udder seemed to be about equally

effective in the two trials in which various treatments were compared. Massage with a damp cloth on one extreme proved just as good as the more drastic preparation involving bathing the udder in hot water on the other extreme. However, since one purpose of the preparation is to provide sanitary conditions for milking, one would not want to discourage the use of hot or warm water in properly preparing the cow for the production of clean milk. Two trials (experiment II) show that some preparation of the cow is necessary to encourage the initial let-down of milk and to shorten the milking time. Merely brushing the udder to remove loose dirt or simply using the strip cup did not prove adequate in stimulating maximum initial let-down.

This work indicates that the rate of milk flow after proper stimulation and with continued relaxation of the cow is dependent upon the size of the teat ducts, condition of teat orifice and strength and relaxation of the sphincter muscles, which is in agreement with the work of Dodd and Foot (3, 4).

#### SUMMARY

The temperature of the udder wash water as used in these trials (45, 100 132° F.) was a minor factor in the stimulation of milk let-down. Proper stimulation of udders at required intervals (1-min. interval as used in these experiments) before milking was necessary for maximum speed of let-down of milk.

A cleaning (massaging) period of 10 to 15 sec. with a cool, damp towel (previously stored in a dry bucket) or a wet towel wrung from water gave the required stimulation for rapid let-down of milk. A similar treatment with the dry hand or the use of a strip cup was inadequate. Bathing udders in hot water (120° F.) for a period of 10 to 15 sec. as a means of pre-milking preparation did not appear to be any more effective than the use of a damp towel. Without pre-milking treatment of the udder, the milking period was prolonged approximately 1 min., as compared with proper preparation and the initial let-down was slow.

Total milk production remained fairly constant throughout these experiments regardless of the method of pre-milking udder treatments.

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## THE EFFECT OF BACTERIA ON THE FERTILITY OF BOVINE SEMEN<sup>1</sup>

L. J. BUSH<sup>2</sup>, T. M. LUDWICK, L. C. FERGUSON AND FORDYCE ELY

Department of Dairy Husbandry, College of Agriculture, and Department of Bacteriology,  
Ohio State University, Columbus

The problems associated with the presence of bacteria in semen have received considerable attention in recent years. Various workers (3, 6, 9, 14) have reported on the number of bacteria found in bull semen collected with an artificial vagina. The number in undiluted semen has been found to range from less than 100 to 22,000,000 bacteria per milliliter and in diluted samples from 200 to 3,400,000 organisms per milliliter. Gunsalus *et al.* (14) have shown that cleaning of bulls materially affects the bacterial count, however.

Foote and Salisbury (11) found more bacteria in the first than in subsequent ejaculates and fewer organisms in the semen from bulls of known high fertility than in the semen from other bulls studied. In contradiction to these findings, Almquist *et al.* (6) found no significant differences between the counts on 91 first and 91 second ejaculates and no significant relationship between fertility and the number of bacteria present in the undiluted semen.

The types of organisms which have been isolated from semen by various workers (9, 10, 14) are *Pseudomonas*, *Streptococcus*, *Micrococcus*, *Bacillus*, diptheroids, coliform organisms, actinomycetes and yeast.

Dondero (9) has pointed out that the sources of bacteria in diluted bull semen are many, *i.e.*, the genital tract of the bull, surface areas, non-sterile diluter, etc.

The possibility of certain bacteria having a detrimental, direct effect on sperm has received some attention. Edmondson *et al.* (10) found that hemolytic bacteria decreased the length of time semen could be stored, whereas certain non-hemolytic organisms increased the storage time from 1 to 4 days over the controls. Gunsalus *et al.* (14) noted that *E. coli* improved the motility of spermatazoa in semen samples into which it was inoculated.

Clinical observations by Williams (22) and Gunsalus *et al.* (14) have indicated that bulls harboring bacteria such as *Streptococcus viridans* or *Pseudomonas aeruginosa* often may have a low breeding efficiency. These and other organisms have been isolated by several workers (13, 14, 23) from bulls that were practically or completely sterile. Similarly, the work of several investigators (7, 21, 22) has shown that certain organisms, many of which frequently are found in semen, often are associated with conditions in cows such as vaginitis, cervicitis, metritis, salpingitis, ovarian bursitis or tubo-ovarian abscesses.

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<sup>2</sup> Present address: Dairy Section, University of Kentucky, Lexington.

In agreement with this, Moore (18) found disease of the tubular genitalia of the male to be associated with these conditions in cows. Many of the same organisms have been reported (8, 15, 23) to be associated with more severe pathological conditions such as abortions and/or retained placenta in cows. Comstock (8), Gilman (13) and Bartlett (7) have reported that organisms of the *Streptococcus* and *Corynebacterium* types were associated with sterility in cows. Hatch *et al.* (16) isolated *Corynebacterium*, *Streptococcus*, *Diplococcus*, *Micrococcus*, *Bacillus* and coliform organisms from the reproductive tract of infertile cows.

The effect of several substances upon the motility of sperm and upon the control of bacteria in semen has been investigated by a number of workers (2, 3, 11, 12). The effect of the addition of some of these substances on fertility also has been investigated. Salisbury and Knodt (19) reported that the addition of 300 mg. per cent of sulfanilamide to semen improved the fertility significantly; however, the beneficial effects were thought to be largely metabolic ones, rather than due to bacterial control alone. Almquist (4, 5) has reported that additions of penicillin, streptomycin or a combination of the two improved the fertility of semen from relatively infertile bulls over untreated controls, whereas sulfanilamide failed to show such improvement. The beneficial effects in the case of penicillin, at least, were attributed to the control of bacteria in the semen. However, Almquist *et al.* (1) previously had reported that penicillin did not affect appreciably the fertility of semen from high fertility bulls.

The purpose of the study reported here was to determine whether the number of bacteria present in diluted semen, as used by the technician, or the predominating types of bacteria occurring in the routine semen samples have any relation to fertility.

#### EXPERIMENTAL METHOD

The material for study included 241 routine samples of diluted semen from 64 bulls in use in two artificial insemination units.<sup>3</sup> This part of the study was conducted in April, 1949. Immediately after collection, the semen was diluted with yolk-citrate diluter to which had been added sulfanilamide at the rate of 300 mg. per 100 ml. of diluter. Plating on blood-agar was done when the semen was approximately 24 hr. old.

Five mg. of para-aminobenzoic acid were added to each 100 ml. of tryptose blood agar base (Difco) to allow the growth of any bacteria which might be susceptible to the sulfanilamide present in the diluted semen. Citrated horse blood was added to this base at the rate of 10 ml. per 100 ml. of agar base. After pouring, and before using, all plates were incubated at 37° C. for 24 hr. to insure the sterility.

The diluted semen was transferred to the blood-agar plates with a sterile pipette and subsequently spread with a glass rod which was dipped in alcohol and flamed between each operation. Tests made to determine whether any bac-

<sup>3</sup> The authors are indebted to the personnel of the Northern Ohio Breeder's Cooperative Assoc. and of the Central Ohio Breeder's Cooperative Assoc., for furnishing the material for study and for assisting in compiling the fertility data for analysis.

teria were being transferred from one plate to another by this procedure showed that the method was satisfactory.

After incubation at 37° C. for 48 hr. the number of colonies on each plate was counted, using a Quebec Colony Counter, and the number of bacteria that would have been present in an entire milliliter of the diluted semen was calculated. Typical predominating colonies were transferred to serum infusion agar slants for further identification. Classification of the bacteria followed the methods described by Merchant (17).

Samples of the diluter used also were examined in a manner identical to that described for the diluted semen. The purpose of this was to determine the extent to which the diluter was the source of the bacteria found in the diluted semen.

Twenty-nine samples of undiluted semen having low motility, or for other reasons not considered good enough for shipment, were plated on blood agar in a manner similar to that described above to determine whether any particular organism consistently was associated with poor quality semen samples.

In a further attempt to determine the source of the various organisms found in the diluted semen, and especially to extend previous observations on the source of *P. aeruginosa* in semen, swabs were taken of the prepuce of nine bulls in use in an artificial insemination unit. These swabs were streaked on blood-agar plates and typical colonies present after 48 hr. incubation at 37° C. were transferred to serum infusion slants for identification.

Analysis of the fertility data was made according to methods described by Snedecor (20).

#### RESULTS AND DISCUSSION

There was a considerable difference between bulls with respect to the range in the number of bacteria per milliliter of diluted semen. Some bulls consistently had low bacterial counts, while other bulls varied considerably from one ejaculate to another in this respect. A difference was found between bulls with regard to the types of bacteria predominating in the diluted semen samples. Some bulls consistently had one type of bacteria in their semen, whereas other bulls were not consistent with respect to the predominant type. This indicates that, in the case of some of the bulls, the bacterial contamination was due to organisms harbored in the genital tract of the bull; in the case of other bulls, the bacteria represented surface contamination.

The number of bacteria per milliliter of diluter ranged from 0 to 8,000 for 14 samples from one association and from 0 to 60 for five samples from the other insemination unit. Large variation from sample to sample indicated that the precautions taken in the preparation of the diluter may influence markedly the number of bacteria introduced in this manner. *Streptococcus*, *Micrococcus* and *Corynebacterium* species were isolated; however, the type of bacteria found in the diluter often was not the predominating type found in the diluted semen samples.

For the purpose of statistical analysis the semen samples were grouped on the basis of the number of bacteria per milliliter. The number of cows conceiv-

ing and the number not conceiving were determined for each respective group. A total of 11,912 first services was considered in the analysis. The criterion of fertility was the failure to return for service during a 60- to 90-day period following insemination. A chi-square test of independence to determine whether there was any relationship between the number of bacteria present in semen samples and their fertility was performed. The results are shown in table 1.

TABLE 1  
*Relation of the number of bacteria to fertility of semen samples*

		No. of bacteria/ml. of diluted semen				
		0-100	101- 1,000	1,001- 10,000	10,001- 100,000	Totals
No. cows conceiving	X = 2,335 m = 2,249	3,448 3,445	1,340 1,393	366 402		7,489
No. cows not conceiving	X = 1,242 m = 1,328	2,032 2,035	876 823	273 237		4,423
Totals	3,577	5,480	2,216	639		11,912
X <sup>2</sup> = 22.94		X <sup>2</sup> .05 = 7.81	X <sup>2</sup> .01 = 11.34			

A highly significant chi-square value ( $P = 0.01$  or less) was obtained. The highly significant deviations from the theoretical values indicate that there is some association between number of bacteria present in diluted semen samples and fertility, since so large a value of chi-square would occur rarely by chance alone. Furthermore, the deviations were consistent in that conception rate decreased as the number of bacteria increased.

The samples also were grouped on the basis of the types of predominating bacteria and 11,803 first services were divided into the respective groups as

TABLE 2  
*Relation of predominating types of bacteria to fertility of semen samples*

		Type of bacteria predominating						
		A <sup>a</sup>	B	C	D	E	F	Total
No. cows conceiving	X = 764 m = 795	518 555	2,443 2,467	773 782	1,505 1,440	1,416 1,380		7,419
No. cows not conceiving	X = 501 m = 470	364 327	1,482 1,458	471 462	786 851	780 816		4,384
Totals		1,265	882	3,925	1,244	2,291	2,196	11,803

\* A = *P. aeruginosa*; B = *P. aeruginosa* and *Corynebacterium*; C = *Corynebacterium*; D = *Micrococcus*, *Streptococcus*, *Diplococcus*; E = Enteric group; F = Negative samples. X<sup>2</sup> = 21.17  
X<sup>2</sup> 0.05 = 11.07      X<sup>2</sup> 0.01 = 15.08.

above. The results of this analysis on the relation of the types of bacteria to fertility of semen samples are shown in table 2. Here also, a highly significant chi-square value ( $P = 0.01$  or less) was obtained, indicating some association between the types of bacteria predominating in diluted semen samples and their fertility. Apparently certain bacteria, i.e., *P. aeruginosa*, *Corynebacterium*, *Streptococcus*, *Micrococcus* and *Diplococcus* tend to have an adverse effect on

conception rate, while the enteric organisms encountered had no such effect. *P. aeruginosa* was apparently the most detrimental. These results may help to explain some of the findings that have been reported on the use of antibiotics such as penicillin and streptomycin on the semen of low-fertility bulls.

To determine whether insemination with semen containing large numbers of bacteria had any effect on the length of the subsequent estrus cycle, the groups described above were compared in this respect. The mean length of the estrus cycle following insemination with semen containing different numbers of bacteria was determined. The small differences which were found between means were not statistically significant, as was shown by an analysis of variance.

A similar analysis was made on the relation of the predominating types of bacteria in the samples to the length of the estrus cycle following insemination. The mean length of the subsequent estrus cycle was determined for each of the groups. An analysis of variance showed that the small observed differences between means were not significant, even at the 5 per cent level of significance. Even though insemination with semen containing large numbers of bacteria, especially of certain types, may affect fertility, these results indicate that the failure of conception occurs without affecting the length of the subsequent estrus cycle.

No obvious relationship was found to exist between the types of bacteria present in the semen and the distribution of the returns following insemination.

*P. aeruginosa* and *Corynebacterium* species were the types of bacteria most frequently predominant in the poor-quality semen samples; however, other types predominated in some cases. Although it is possible that some of the bacteria isolated from these samples had some casual relationship to the quality of semen produced, the fact that the same types were found in high quality semen made it difficult to draw any conclusions from these observations.

*P. aeruginosa* was isolated from both the preputial orifice and the preputial cavity of bulls. Attempts to isolate the organism from some bulls which consistently had shown it in their semen were unsuccessful. On the other hand, the organism was isolated from some bulls which had never shown it in their semen.

#### SUMMARY AND CONCLUSIONS

A significant relationship was found between the number of bacteria in diluted semen and its fertility when all types of bacteria were considered.

A significant relationship was found between the types of bacteria predominating in the diluted semen and its fertility. Types such as *Corynebacterium*, *Micrococcus*, *Streptococcus* and *Diplococcus* species and especially *P. aeruginosa*, tend to have an adverse effect on conception rate, whereas the enteric organisms encountered had no such effect.

No relationship was found between either the number of bacteria or types of bacteria predominating in diluted semen and the average length of the estrus cycle following insemination.

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# THE DEVELOPMENT OF CALVES RAISED WITHOUT PROTOZOA AND CERTAIN OTHER CHARACTERISTIC RUMEN MICROORGANISMS

W. D. POUNDEN<sup>1</sup> AND J. W. HIBBS

*Department of Dairy Industry, Ohio Agricultural Experiment Station, Wooster*

During investigations concerning the establishment of rumen function in calves, the growth and development were observed of a limited number of animals from birth to 6 mo. or more of age whose rumens were maintained free of usual varieties of rumen microfauna and certain characteristic microflora. Although the importance of rumen microorganisms in ruminant digestion has long been recognized, there is but limited information regarding the effects on young cattle of the absence of these microorganisms.

Protozoa from the rumen, originally described by Gruby and Delafond (9), were summarized by Mangold (14) under more than 30 species. Apparently, all or most of these have been found in the rumens of cattle in North America (5). A complete understanding of their role in the digestive economy of the host animals is lacking, notwithstanding numerous investigations. From their observations of the numbers present and their later destruction in the abomasum, Ferber and Winogradowa-Fedorowa (8) concluded that they had an essential role in the development of the host animals. Mangold (15) states that the proportion of food protein metabolized by infusoria and subsequently digested by the host is considerable. According to Baker (1), protozoa operate as agents in the removal of iodophile microorganisms and so contribute to the maintenance of a balanced population. Hungate (12, 13) found that certain *Diplodinium* species could digest cellulose to some extent. Their presence is considered of little importance by other investigators because sheep and goats can get along without them (3, 4, 6).

Becker (3) noticed that lambs, experimentally defaunated with  $\text{CuSO}_4$  solutions and starvation, tended to show rotundity of the body as if somewhat bloated when fed alfalfa and ground grain. Calves raised in partial segregation on rations of limited quantities of milk and alfalfa hay alone failed to develop usual rumen varieties of protozoa and certain characteristic indicator rumen bacteria during their first 6 wk. of age (17). They appeared to have rougher hair coats than similarly fed calves which received rumen inoculations. The abdomens of the uninoculated calves also appeared to be deeper than in the inoculated calves. During their first 6 wk. of age, this "pot-bellied" condition was not apparent in other uninoculated calves which received grain (17) or had access to lawn pasture (20). One uninoculated calf which was 2 mo. old when turned on pasture developed a noticeably rougher hair coat than similarly treated but inoculated calves, and it suffered from recurrent mild diarrhea during the 7-wk. experimental period.

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<sup>1</sup> Present address: Bureau of Animal Industry, U.S.D.A., Beltsville, Md.



## EXPERIMENTAL

Four uninoculated Jersey calves were raised from birth in segregation until they were 6 mo. old and one of them up to 8 mo. of age. Controls were provided by similar calves which received similar feeds from the same lots and which also were given rumen inoculations with cud materials (17, 19).

The calves were fed whole milk at the rate of 0.9 lb. per 10 lb. body weight at birth per day for the first 6 wk. and half this amount during the seventh week. No milk was fed after the seventh week. Fairly good quality alfalfa hay was provided free choice from birth throughout the experimental period. A simple 14.5 per cent protein grain ration consisting of corn, oats, bran and soybean oil meal was added to the ration when the calves were 6 wk. old. They received it in the proportion of half the quantity of hay they were consuming.

Rumen samples were collected repeatedly by stomach tube and examined in the manner described previously (17) for the presence of usual rumen protozoa and certain characteristic rumen microflora used as indicators of the presence of usual rumen bacteria.

## RESULTS

All four calves still lacked usual rumen protozoa at 6 mo. of age and one as late as 8 mo. They all developed large coccoid bacteria, previously designated as making up hay-flora group I (17), in their rumens between the ages of 1 and 2 mo. One calf became inoculated at 9 wk. of age with the large cigar-shaped organism (probably *Oscillospira*) of hay-flora group II (17). This occurred by accident through contact with unsterilized equipment which had been used previously on inoculated calves. Various rumen microflora other than those being used as indicators of the presence of usual rumen microorganisms probably were transferred at the same time. The highest relative concentrations of this large cigar-shaped microorganism ever encountered were in rumen samples from this calf. Limited numbers of the same organism were present in rumen samples from a second calf in the adjoining pen beginning at 4 mo. of age but never were observed in samples from the other two. This microorganism has been observed to disintegrate readily in abomasal fluids, such as occurs in the case of rumen protozoa (2, 16). The remaining two organisms composing hay-flora group II (17), namely the small rods in flat rectangular groups and the thick square-ended rods, never were observed in samples from these four segregated calves.

The average weight of these four calves at 6 mo. of age was 229 lb. as opposed to the 235.5-lb. average of the 12 inoculated calves. Thus, there was only an average difference of 5.5 lb. between the groups.

The calf (fig. 1) having the cigar-shaped organisms present in its rumen was of good appearance both as to hair coat and condition, although possibly it was a little paunchy in comparison with the inoculated calves (fig. 2). The hair coats of the other three calves (fig. 3) appeared rough and they were not as well conditioned as the calves in the inoculated group. They had visibly deeper abdomens that gave them a "pot-bellied" appearance.

The partly digested feed present in two rumens devoid of protozoa, one of



FIG. 1. Partial rumen-inoculated calf (6 mo. old). Rumen devoid of usual rumen protozoa but containing large cigar-shaped rods of hay-flora group II.

which belonged to the calf having considerable numbers of the large cigar-shaped organisms present, was not visibly distinguishable from that present in the rumens of inoculated calves receiving similar feeds.

Uninoculated and segregated calves frequently were noticed to nose down through the bedding and to pick up and eat pieces of wet straw and chaff.



FIG. 2. Rumen-inoculated control calf (6 mo. old). Rumen contained characteristic microorganisms.

Although three of these calves persistently did this, the calf having the cigar-shaped organisms in its rumen seldom was observed to do so.

#### DISCUSSION

These results obtained with calves devoid of characteristic rumen protozoa further support the opinion that rumen protozoa are not essential to the host animals. However, it must be kept in mind that the experiments so far carried out for an extended period of time with lambs (3) and calves have been under conditions of grain feeding and far removed from those that would exist under primitive conditions. Consequently, more extensive experiments may yet demonstrate a real function and value of protozoa, *e.g.*, as predigestors of undigestible bacteria, if the diet excessively stimulates these. The defaunating treatment used in the experiments mentioned by Becker (3) may have eliminated certain varieties of characteristic microflora besides the protozoa. Thus, the rotundity of their lambs may have been due to reasons similar to those causing the deeper, "pot-bellied" appearance of our calves.



FIG. 3. Uninoculated calf (6 mo. old). Rumen devoid of usual rumen protozoa and hay-flora group II.

Support is provided for the idea that microorganisms that have developed over a period of time in the environment of the rumen would be more likely to function most efficiently in this organ by: (a) the clinical manifestations of rough hair coats and deep, "pot-bellied" middles observed in these uninoculated and segregated calves when compared with inoculated calves on similar fairly high roughage rations; (b) the finding of clinical cases in the field which apparently respond to treatment with rumen inoculations (19); (c) the observed differences in blood plasma ascorbic acid levels between young inoculated and uninoculated calves on milk and hay rations (10); and (d) differences in similar groups of calves in their ability to digest cellulose (7).

A possible explanation for the tendency for the uninoculated calves to nose down into the bedding is that some natural instinct caused them to seek in such locations for substitute microorganisms to assist in carrying on the functions of their rumens. It was interesting to note that the one calf of the four animals whose rumen was devoid of protozoa, but which had the large cigar-shaped bacteria present in large numbers and presumably various other usual rumen microflora, had much less tendency to do this.

Observations such as those reported by Udall (21, 22) that association of calves with nurse cows promotes a more satisfactory condition of health in the calves possibly may be explained in part on the basis of improved transfer of rumen microorganisms. It also is quite possible that calves are stimulated by example to eat more roughage when along with cows. This would be of assistance in promoting an early establishment of usual rumen microorganisms (17, 18).

With reference to rumen inoculations with cud materials as a preventive or cure for abnormal conditions in cattle, Hofflund (11) says the practice was used as much as 100 yr. ago in Sweden. Cuds which were obtained from cattle in other districts were given to cattle which had become debilitated due to existing on forage from an area in which the crops were deficient.

#### SUMMARY

The growth and development of four Jersey calves which were raised in pens segregated from other cattle were compared with 12 others which were inoculated with cud material from older cattle and raised at the same time on similar rations of alfalfa hay and limited quantities of grain.

The uninoculated calves failed to develop usual protozoa in their rumens and also some varieties of characteristic rumen microflora which were used as indicators of the presence of usual rumen microorganisms. One of the four calves accidentally received a partial rumen inoculation. This resulted in one type of the characteristic indicator microflora which readily is digested by abomasal fluids becoming established in its rumen.

Average gains in weight at 6 mo. of age were 229 lb. for the four uninoculated calves and 235.5 lb. for the 12 inoculated animals, a difference of only 5.5 lb. The calf which received the partial rumen inoculation had a neat and healthy appearance similar to the control inoculated calves, but the hair coats of the other three were much rougher in appearance. Their abdomens seemed deeper and "pot-bellied." The latter three had a persistent habit of nosing down through the bedding to pick up wet bits of straw. It was considered possible that this habit was due to a stimulus to seek inoculation of their rumens with substitute rumen microorganisms in the absence of the usual microflora and fauna.

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# RATE OF ABSORPTION OF CAROTENE AND OF VITAMIN A FROM THE ALIMENTARY TRACT OF DAIRY CALVES.

## I. EFFECT OF METHOD OF ADMINISTRATION<sup>1</sup>

N. L. JACOBSON, G. H. WISE<sup>2</sup>, R. S. ALLEN AND O. KEMPTHORNE

*Iowa State College, Ames*

The importance of vitamin A activity in the nutrition of dairy calves has continued to focus attention on the quantitative dietary needs for carotene and vitamin A. The establishment of optimal allowances of these nutrients is contingent upon a knowledge of factors affecting utilization. Among the multitude of variables that may be related to efficiency of absorption and utilization are the vehicle of the vitamins, the dispersion of the vitamin concentrate and the methods of administration. Of these, only the last will be considered herein.

Lemley *et al.* (5) observed that when vitamin A in an oil medium was injected either subcutaneously or intramuscularly the effectiveness was 35 per cent and 2 per cent, respectively, as great as when taken *per os*. Water-solubilized carotene given intramuscularly, however, was utilized efficiently by rats (11). Aqueous dispersions of vitamin A also were utilized effectively when injected intramuscularly into children (4).

Niedermeier *et al.* (8) found that injections of an aqueous dispersion of vitamin A into the small intestine of the goat effected higher blood plasma levels of this vitamin than did similar injections into either the abomasum or the large intestine. Moreover, when vitamin A was injected into the small intestine of sheep (1), the rate of absorption was more rapid than when placed into the rumen or administered orally. There was little absorption of either carotene or vitamin A from the cecum and the colon.

Since feeding vitamin A and carotene concentrates to calves at different stages of development involves managerial problems as well as nutritional consequences, the objective of this investigation was to compare effects of various methods of administering (nipple feeder, stomach tube and gelatin capsule) supplements on the rates of absorption.

### GENERAL EXPERIMENTAL PROCEDURES

*Experimental subjects, feeding and management.* Dairy calves representing four different breeds, Brown Swiss, Guernsey, Holstein and Jersey, were used in carotene and vitamin A absorption tests. During the first 3 days following birth, each calf received colostrum from its dam. Subsequently, either fresh whole milk or reconstituted milk was fed twice daily at the rate of 10 lb. per day per 100 lb. body weight of calf. The routine method of feeding was from a nip-

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<sup>2</sup> Present address: Department of Animal Industry, North Carolina State College, Raleigh.

ple pail. At various stages of growth of several of the calves, a concentrate mixture and hay were incorporated in the diet. All the experimental subjects were confined to individual pens bedded with wood shavings. Whenever calves were restricted to milk diets, the individuals were muzzled to minimize consumption of foreign material.

*Carotene and vitamin A supplements.* In most of the trials the source of carotene was "Carex"<sup>3</sup>, a carrot oil that contained 5,000 I. U. of carotene per gram, but during the terminal stages of the investigation, carotene in cottonseed oil<sup>4</sup>, 50,000 I. U. per gram, was administered. The source of vitamin A was fish liver oil concentrates. The potency of the product used during the early trials was 25,000 I. U. per gram<sup>5</sup>, whereas that given in the later studies was 30,000 I. U.<sup>6</sup>.

The quantity of supplement given in absorption tests was 1,000 I. U. per lb. of body weight. The measured amount for each subject was administered either dispersed (by homogenization) in milk or enclosed in gelatin capsules. The milk-dispersed supplement was given either orally from a nipple feeder or intraruminally through a stomach tube. Milk fed from a nipple normally traverses the esophageal groove and enters the abomasum directly (12). In the stomach-tube method of administration the supplements dispersed in milk were passed through a horse catheter into the rumino-reticular cavity. The volume of fluid used for the dispersion medium was approximately the same for either system, nipple or tube. It is possible, however, that in some instances the volume administered by tube might have exceeded the capacity of the rumen and reticulum, thus resulting in an overflow into the abomasum. A balling gun was used to administer the capsules, special care being taken to avoid their rupture before swallowing. Capsules thus administered would be expected to pass into the rumen.

*Blood collection and analytical procedures.* The criteria of the rates of absorption of carotene and of vitamin A were the levels of these substances in samples of plasma from venous blood collected at the time of feeding and at 2, 4, 8, 12 and 24 hr. thereafter. Blood plasma carotenoids and vitamin A were determined by procedures described by Squibb *et al.* (10).

#### TRIALS AND RESULTS

*Trial I—Nipple feeder vs. stomach tube.* At intervals of approximately 1 wk., the milk normally given at the morning feeding was replaced with reconstituted separated milk in which either a carotene or a vitamin A concentrate had been dispersed. The nipple and the stomach-tube methods of administration were alternated from period to period for each calf. In these comparisons eight animals received carotene supplements and six, vitamin A.

The mean pre-absorption carotenoid and vitamin A values in blood plasma are shown in table 1 (trial I). Each initial value was considered as the base

<sup>3</sup> Obtained from Nutrition Research Associates, South Whitley, Ind.

<sup>4</sup> Obtained from General Biochemicals, Inc., Chagrin Falls, O.

<sup>5</sup> Obtained from White Laboratories, Inc., Newark, N. J.

<sup>6</sup> Supplied by the Borden Co., New York, N. Y., courtesy of L. T. Wilson.

level (zero) from which subsequent changes were determined. Mean responses to the respective methods of administering the supplements are depicted in figures 1 and 2.

Although the magnitude of the increases resulting from each method of administration was variable in the different calves and in the same calf at various periods, the nipple system uniformly resulted in more rapid rises than did the stomach-tube procedure. The differences in the levels of carotenoids were more pronounced than those of vitamin A. The maximum values for vitamin A, however, were attained more quickly than those for carotenoids.

TABLE 1  
*Mean pre-supplementation concentrations of carotenoids and vitamin A  
in the blood plasma of calves*

Trial	Supplement	Method of administration	Mean level in blood plasma	
			Carotenoids	Vitamin A
(γ/100 ml.)				
I	Carotene in oil	Nipple feeder	15.2	11.7
		Stomach tube	18.9	11.6
	Vitamin A conc.	Nipple feeder	19.6	13.1
		Stomach tube	16.5	12.5
II-a	Carotene in oil	Nipple feeder	19.8	7.4
		Capsule	17.0	6.5
	Vitamin A conc.	Nipple feeder	14.2	9.2
		Capsule	13.6	8.2
II-b	Carotene in oil	Nipple feeder	57.3	16.6
		Capsule	60.0	17.8
	Vitamin A conc.	Nipple feeder	35.0	15.7
		Capsule	42.4	15.7

Small but relatively uniform increases in concentrations of vitamin A in the blood plasma occurred during at least the first 12 hr. following the administration of carotene (fig. 1). Even though the carotenoid level was higher at 24 hr. than at 12, the vitamin A concentration was lower. The true relationship between the values of these constituents in plasma is obscure. Following vitamin A absorption (fig. 2), the carotenoid values in the plasma decreased, the degree and rate of depression being somewhat greater from the nipple administration than from the stomach tube.

In the absence of any well-established law relating concentrations of vitamin A and of carotenoids in the blood plasma to time after feeding massive doses of these substances, it was decided, for the purpose of statistical analysis, to obtain the average (or linear) rate of increase of concentration over the period



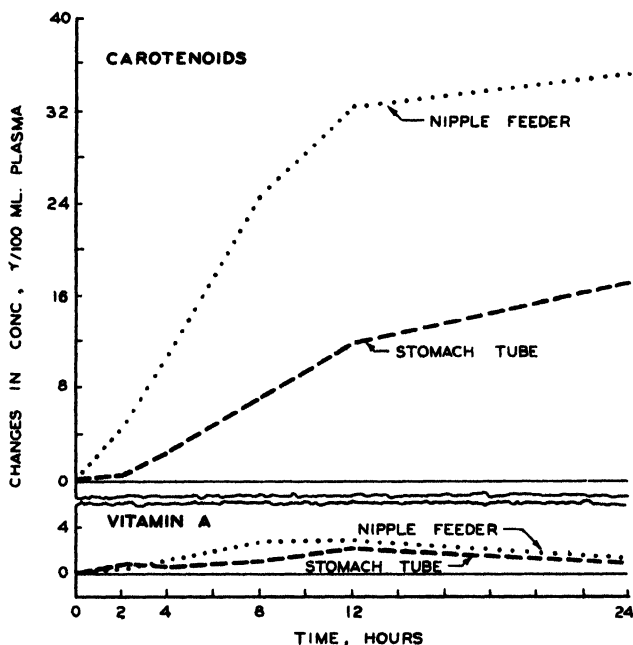


FIG. 1. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight calves that received massive doses of a carotene concentrate homogenized in milk and administered by nipple feeder and by stomach tube.

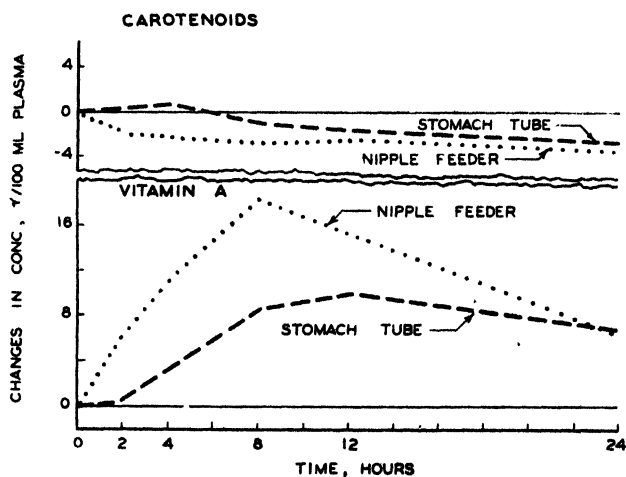


FIG. 2. Mean changes in levels of carotenoids and vitamin A in blood plasma of six calves that received massive doses of a vitamin A oil concentrate homogenized in milk and administered by nipple feeder and by stomach tube.

0 to 12 hr. The curvature in this relationship was examined by evaluating the quadratic component orthogonal to the linear component. If the increases at 2, 4, 8 and 12 hr. are denoted by  $I_2$ ,  $I_4$ ,  $I_8$  and  $I_{12}$ , the linear rate of uptake  $L$  and the orthogonal quadratic component  $Q$  are apart from constant numerical divisors, thus

$$L = -8I_2 - 3I_4 + 7I_8 + 17I_{12}$$

$$Q = -20I_2 - 109I_4 - 113I_8 + 115I_{12}$$

The  $L$  and  $Q$  values were appraised by a simple analysis of variance. The variations in these values were separated into those due to differences between calves, those due to differences between treatments and those due to treatment by calf interactions. The significance of treatment effects on either  $L$  or  $Q$  was determined by comparing the average effect with a variance which measures the failure of the effect to be the same for all calves. Thus, the test of significance was made by comparing the mean square for treatment with the mean square for calf-treatment interactions.

The analysis of the  $L$  values for the first set of data, table 2, revealed a dif-

TABLE 2

*Analysis of variance of linear changes of blood plasma carotenoid levels of eight dairy calves following administration of massive doses of carotene homogenized in milk*

Source of variation	Degrees of freedom	Sums of squares	Mean square
Calves	7	609,260	87,037
Treatments (Nipple pail versus stomach tube)	1	682,235	682,235
Treatments $\times$ calves	7	503,927	71,990
$F = \frac{682,235}{71,990} = 9.28^a$			

<sup>a</sup> Significance  $P_{.05} = 5.59$

$P_{.01} = 12.25$

ference significant at the 5 per cent level in the linear rates of increase of blood plasma carotenoid levels following administration of carotene by the two methods. A like analysis of the corresponding  $Q$  values also showed a difference significant at the 5 per cent level of probability.

Although the remaining data were analyzed in a manner similar to those illustrated in table 2, only summary statements are presented.

In this first trial the data on vitamin A uptake during the initial 12 hr. following administration of this vitamin showed a difference in curvatures that approached significance at the 5 per cent level, whereas the differences in the linear components were non-significant. This was due, in part, to the marked downward trend in the nipple-fed group after the eighth hour.

**Trial II. Nipple feeder vs. capsule.** The experimental subjects were 60-day old calves that had been used in a previous study (7) in which all subjects were restricted to a fortified filled-milk diet. Since the calves had not consumed solid feed, it was assumed that the rumen was underdeveloped. To gain

information on the effects of diet and/or rumen development, two series of absorption trials were conducted: the first, while the animals were on a milk diet and, the second, after 2 mo. on a conventional milk, concentrate and hay regime.

a. *Whole milk diet.* The diet of the calves was changed from the filled milk to whole milk. Subsequently, the animals were divided into two units: group A consisted of eight Holsteins and two Guernseys and group B, seven Holsteins and one Guernsey. Each group was divided further into two subgroups (table 3). At approximately weekly intervals, carotene and vitamin A supplements were given at the rate previously indicated. The methods of administration were as outlined in table 3.

The initial values of carotenoids and of vitamin A in blood plasma are shown in table 1 (trial II-a) and the responses to supplementation in figures 3 and 4. The rate of carotene (oil concentrate homogenized in milk) absorption (fig. 3) following ingestion from the nipple feeder was similar to that

TABLE 3  
*Grouping of calves and plan of administering supplements (Trial II)*

Supplements <sup>a</sup>	Group	Sub-group	No. of calves	Method of administration	
				Period I	Period II
Carotene in oil (carotene)	A	1	5	Nipple feeder	Capsule
		2	5	Capsule	Nipple feeder
Fish liver oil (vitamin A)	B	1	4	Nipple feeder	Capsule
		2	4	Capsule	Nipple feeder

<sup>a</sup> Administered at rate of 1000 I. U./lb. body wt.

in trial I (fig. 1) but more rapid than when the oil was given in a capsule (fig. 3). The differences in linear trends resulting from the two methods of administration were significant at the 1 per cent level, but the differences in curvatures were non-significant statistically.

The rate of absorption of vitamin A (fig. 4) was somewhat greater when the fish liver oil concentrate was fed from a nipple than when given in a capsule, but in the former the maximum level was attained at approximately 12 hr. after ingestion, whereas in the latter the maximum occurred later. During the initial 12 hr., the difference in the linear trends of vitamin A in blood plasma of calves receiving the supplement by the two methods was statistically significant at the 5 per cent level, but the difference in curvatures of rates of uptake was not significant.

When the nipple system of feeding was employed, the corresponding responses in trials I and II-a to carotenoid intake (figs. 1 and 3) and to vitamin A (figs. 2 and 4) were strikingly similar. A comparison of the stomach-tube method (figs. 1 and 2) with the capsule procedure (figs. 3 and 4) indicates that the rate of absorption of the supplements was more rapid when the former of the two methods was used. Moreover, the extent of carotenoid suppression in the blood plasma following vitamin A supplementation was somewhat greater in trial I than in trial II-a.

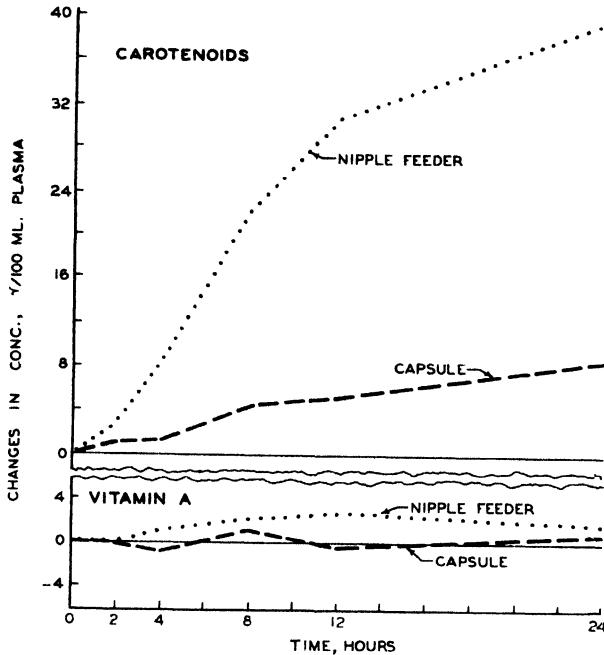


FIG. 3. Mean changes in levels of carotenoids and vitamin A in blood plasma of ten 2-mo.-old calves that received a basal diet of whole milk and a supplement of massive doses of carotene administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

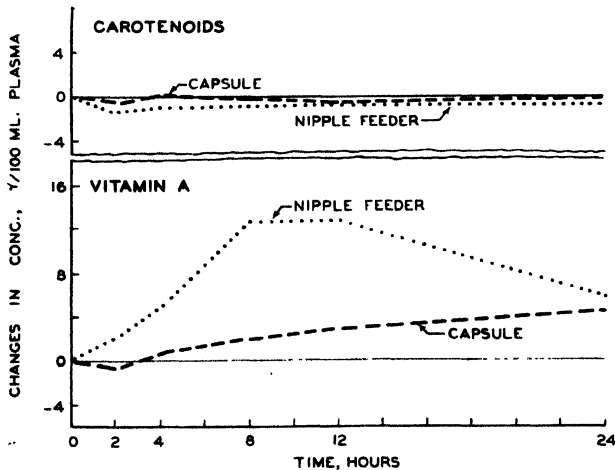


FIG. 4. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight 2-mo.-old calves that received a basal diet of whole milk and a supplement of massive doses of vitamin A administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

b. *Buttermilk (reconstituted), concentrate mixture and alfalfa hay diet.* After this diet was fed to the same calves employed in trial II-a (less one calf in carotene group) for a period of 2 mo., the plan of administering carotene and vitamin A, table 3, was repeated. Since the dry separated milk available was more readily reconstituted and, thus, was a more desirable dispersion medium for the supplement than the dry buttermilk commonly fed, the former was substituted for the latter when the vitamin substances were administered. Other components of the diet, concentrate mixture and hay, were unchanged on the day of the tests.

As a result of hay consumption, the base levels of carotenoids and of vitamin A in the blood plasma of the calves were higher in trial II-b than in II-a (table 1). The post-supplementation changes from these bases are shown in figures 5 and 6.

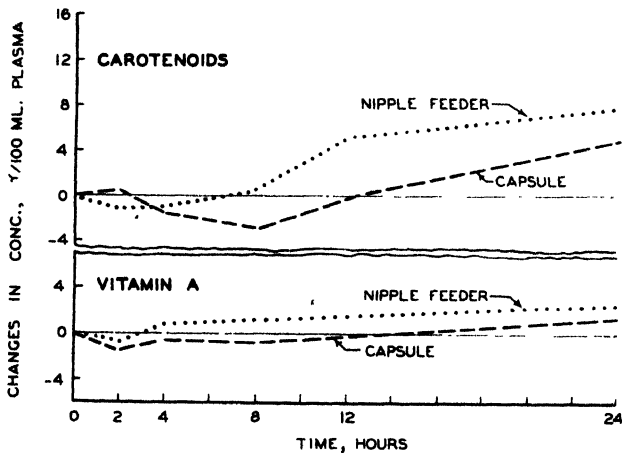


FIG. 5. Mean changes in levels of carotenoids and vitamin A in blood plasma of nine 4-mo.-old calves that received a basal diet of reconstituted buttermilk, alfalfa hay and a concentrate mixture and a supplement of massive doses of carotene administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

The values of plasma carotenoids following carotene administration were slightly greater when the supplement was fed from a nipple than when given by a capsule (fig. 5). During the first 12 hr., the difference in linear trends approached significance at the 5 per cent level, but the difference in curvatures was non-significant. The striking features of the responses in this trial, in comparison with those in trial II-a (fig. 3), were the delayed increases and the subsequent low magnitude. Although, as in preceding trials, the accompanying increases of plasma vitamin A were slight, the higher level of vitamin A corresponded to the higher values for carotenoids.

In contrast to the exceptionally slow rise in carotenoid concentrations in the blood plasma (fig. 5), the increase of vitamin A was rapid (fig. 6). The rate of uptake of this vitamin and the level reached were even greater in this trial than in the preceding (fig. 4). In accord with observations in other trials, vita-

min A was absorbed more rapidly when the nipple procedure of administration was employed than when the capsule method was used (fig. 6). The difference in linear trends, however, during the period from 0 to 12 hr. was not significant, largely due to the precipitous drop in the "nipple" curve after the eighth hour. On the other hand, the difference of curvatures was significant at the 1 per cent level.

A further comparison of responses during the liquid (trial II-a) and the solid (trial II-b) dietary regimes indicates that when vitamin A concentrates were given by capsule, the concentration of this vitamin in the blood plasma was greater in the former trial (fig. 4) at 24 hr. after administration than at

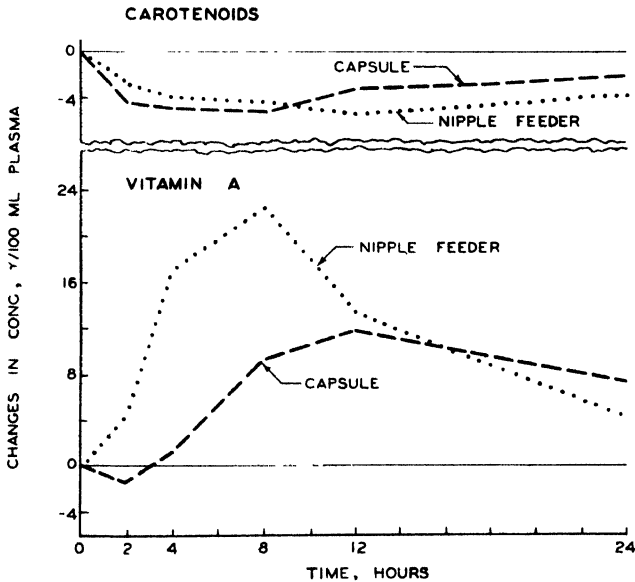


FIG. 6. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight 4-mo.-old calves that received a basal diet of reconstituted buttermilk, alfalfa hay and a concentrate mixture and a supplement of massive doses of vitamin A administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

12 hr., whereas in the latter (fig. 6) the converse was true. This difference in time suggests a more rapid passage of the supplement in the animals having the greater ruminal activity. In trial II-b the depression of carotenoids following vitamin A administration was greater than in trial II-a.

#### DISCUSSION

Although the concentration of any nutrient in the blood at a given time involves many metabolic processes, the results reported herein seem to indicate a relationship between the methods of administering carotene and vitamin A and the rate at which these substances are absorbed from the alimentary tract of dairy calves. There are several possible explanations for the difference ob-

served when vitamin substances were administered by stomach tube and by nipple. Milk ingested by this latter procedure is mixed with relatively large quantities of oral and esophageal secretions (14). This exposure of the dispersed supplements might have evoked physical and chemical alterations that enhanced subsequent absorption. Moreover, the slower rate of uptake of vitamin supplements following stomach-tube administration may be ascribed to their gradual passage from the rumino-reticular cavity and thence into the other stomach compartments and the small intestine. Inasmuch as it has been demonstrated (2) that vitamin A in oil is absorbed in the bovine largely through the lymph of the small intestine, the rapidity with which this absorptive area was contacted by the vitamin substances used in the present experiment might have affected the rate of transmission to the blood. This delay in the fore part of the digestive tract conceivably also could have resulted in an increased loss of potency of the supplements.

Since the carotene and the vitamin A administered in gelatin capsules presumably passed into the rumino-reticular cavity, the retarded rate of absorption probably resulted, in part, from factors similar to those affecting uptake of vitamin substances dispersed in milk and administered by stomach tube. As the rate of uptake in the latter instance was somewhat more rapid, it would seem that absorption might have been enhanced by dispersion of the vitamin supplements. Frazer and associates (3) found that the average particle size of ingested triglyceride fats in the intestine of the rat is less than  $0.5\ \mu$  and that paraffin, which normally does not pass through the intestinal wall, is absorbed when similarly dispersed. Since it has been shown (9) that fats and vitamin A are absorbed in a like manner, it seems possible that vitamin A uptake, like fat absorption, may be influenced by dispersion. The need for further experimentation, however, is indicated since Lundback and Maaløe (6) were unable to confirm the paraffin absorption observations.

The marked reduction in rate of uptake of carotene following the transition from a diet of whole milk to one composed of reconstituted buttermilk, hay and concentrates is difficult to interpret. Possibly the relatively high initial blood plasma carotenoid values of calves in trial II-b (solid diet) might have masked the effects of supplemental carotene. It would seem, however, that this apparent reduced rate of absorption might have been due, in part, to changes in the amount and the type of oil in the carotene supplement and to the quantity of fat in the milk in which the concentrate was dispersed. It is possible that the reduced absorption might have resulted not from any single factor but rather from the combined effect of several of the foregoing.

The relationship between blood plasma values for vitamin A and those for carotenoids following the administration of massive doses of carotene is obscure. Maximum vitamin A levels, subsequent to carotene administration, usually were reached earlier than the corresponding carotenoid maxima. Since the changes in vitamin A values were small, additional experimentation is necessary before this relationship can be clarified.

The maximum blood plasma levels of the vitamin substances fed were at-

tained earlier after vitamin A administration than after carotene feeding, thus suggesting a possible difference in the metabolism of these materials. Since the rates of administration of these substances were similar on the I.U. basis, the quantity of carotene, in micrograms, was greater, thus possibly affecting the time required for maximum levels to be attained.

The 24-hr. experimental period employed in this investigation was too brief to characterize the entire absorption curves. Limited data (13), however, indicate that the blood plasma carotenoid and vitamin A levels following administration of carotene by stomach tube and by capsule increase over a longer period of time and decline more gradually than those resulting from nipple pail feeding. Studies of this nature, even though conducted over an extended interval, may not indicate the efficiency of utilization of vitamin supplements fed by the various methods. Whether a relationship exists between rate of increase in the blood and total absorption remains to be determined by further experimentation.

#### SUMMARY

Carotene and vitamin A given at the rate of 1000 I.U. per lb. of body weight of calf were administered, respectively, by nipple feeder, stomach tube and gelatin capsule.

Comparisons of initial blood plasma carotenoid and vitamin A levels with those 2, 4, 8, 12 and 24 hr. after feeding the vitamin substances were employed as criteria of the rates of absorption.

Carotene and vitamin A dispersed in milk by homogenization and fed by nipple were absorbed more rapidly than similar preparations administered by stomach tube. The rates of absorption of carotene and of vitamin A from concentrates administered by gelatin capsules were somewhat less rapid than those resulting from the foregoing procedures.

The rate of absorption of vitamin A by calves restricted to whole milk was less rapid than the rate of uptake by the same calves after having received a diet of reconstituted buttermilk, hay and grain concentrates for approximately 8 wk. Conversely, the rate of absorption of carotene was more rapid under the former dietary regime than under the latter.

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## DEHYDRATED SWEET POTATOES AS A SUBSTITUTE FOR CORN-SOYBEAN SILAGE

L. L. RUSOFF, G. D. MILLER, B. J. BURCH, JR. AND J. B. FRYE, JR.

*Dairy Department, Louisiana State University, Baton Rouge*

During the winter months when pasture may be poor or unavailable, silage may constitute a vital part of the dairy cow's ration. Many dairymen in Louisiana and in other parts of the South do not have silage or have herds too small for practical silage feeding. Therefore, the question was raised as to whether a suitable home-grown substitute could replace silage. Some dairy farmers in Louisiana have reported that milk production did not fall when sweet potatoes were used during the winter months when no pasture or silage was available.

Sweet potatoes are plentiful at certain seasons of the year and can be stored after dehydration. It is well recognized that dehydrated sweet potatoes are approximately 90 per cent as valuable as yellow corn meal as a source of carbohydrate in the grain ration for dairy animals (1, 2, 4). Rusoff *et al.* (4) also reported that dehydrated sweet potatoes were approximately 17 per cent more valuable than ground snapped corn including cob and shuck for milk production.

Although dehydrated sweet potatoes are classified as a concentrate, it was decided to compare this material with silage for lactating cows.

### EXPERIMENTAL

This study was conducted during the winter months of 1948-1949 and 1949-1950. Dehydrated standard sweet potatoes and dehydrated weevily sweet potatoes (fed wet) were compared with corn-soybean silage for milk production. In a palatability trial using dehydrated "infected" and weevily sweet potatoes, Rusoff and Miller (3) found that animals would consume these culled potatoes as readily as the standard potatoes. Therefore, dehydrated weevily sweet potatoes also were used to determine whether they might affect milk production. Some pasture was available during the 1948-1949 trial, and none in the 1949-1950 trial.

*Trial 1.* A Latin-square design was used. Three groups of eight milking cows each, (five Holsteins and three Jerseys) were given an 18 per cent protein grain mixture according to production. The animals in each group were similar as to age, production and number of previous lactations. Approximately 8 lb. of alfalfa hay per cow per day were fed. Equal amounts of corn-soybean silage, dehydrated standard sweet potatoes or dehydrated weevily sweet potatoes on an air-dry basis were fed to the groups. Approximately 1 lb. of dehydrated sweet potatoes was equivalent to 1 lb. of air-dry silage. This amounted to approximately 9 lb. of dehydrated sweet potatoes or 30 lb. of fresh silage daily. The trial consisted of three experimental periods of 20 days each with a 5-day change-

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over between periods. The milk produced by each cow was weighed daily and tested for butterfat every 10 days during each period.

The amount of 4 per cent fat-corrected milk (F.C.M.) per cow per day produced by each group while fed silage or dehydrated sweet potatoes is given in table 1. The average daily 4 per cent milk production of all groups was 24.4 lb. on the silage, 25.6 lb. on dehydrated standard potatoes and 26.1 lb. on dehydrated weevily sweet potatoes.

TABLE 1

*Pounds of 4% F. C. M. per cow per day produced by the various groups while fed silage or sweet potatoes*

Group	Preliminary period (7 d.)	Corn-soybean silage (20 d.)	Dehydrated standard sweet potatoes fed wet (20 d.)	Dehydrated weevily sweet potatoes fed wet (20 d.)
	(lb.)	(lb.)	(lb.)	(lb.)
A	25.8	24.0	25.2	27.3
B	25.0	24.1	26.6	26.5
C	25.9	25.3	25.2	24.7
Av.	25.6	24.4	25.6	26.1

Table 2 presents the actual feed consumption of silage and dehydrated potatoes on the air-dry basis for each group during each period of 20 days. Any refusals were weighed back. The average amounts of feed consumed were practically the same.

TABLE 2

*Feed consumption of silage and dehydrated sweet potatoes for each group on an air-dry basis*

Group	Silage	Dehydrated standard sweet potatoes	Dehydrated weevily sweet potatoes
	(lb.)	(lb.)	(lb.)
A	1,328.6	1,374.0	1,336.6
B	1,229.0	1,360.0	1,370.4
C	1,390.0	1,380.0	1,382.4
Av.	1,316.0	1,371.3	1,363.1

*Trial 2.* In this study (1949-1950) a double reversal plan was used. Two similar groups of nine animals each (five Holsteins and four Jerseys) were used. The same feeding program and length of periods were followed as in the 1948-1949 test. Dehydrated sweet potatoes consisting of an equal mixture of standard and weevily potatoes which were stored for 1 yr. were compared with corn-soybean silage for milk production. Approximately 9 lb. of dehydrated sweet potatoes or 30 lb. of silage were fed per cow per day so that the air-dry amounts were equalized.

Table 3 presents the 4 per cent F.C.M. production per cow per day for the two groups on the dehydrated sweet potatoes or silage for each period. The average milk productions per cow per day for both groups of animals when fed dehydrated sweet potatoes or silage were 21.5 and 22.3 lb., respectively.

TABLE 3

*Amount of 4% F. C. M. per cow per day during each period of trial 2*

Feed	Preliminary period (10 d.)	Period (20 d. each)			
		1	2	3	Av.
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Dehydrated sweet potatoes	22.6	23.1	21.7	19.6	21.5
Silage	21.4	24.5	21.1	21.3	22.3

The amounts of silage and dehydrated potatoes (air-dry basis) eaten during each period were very comparable (table 4).

The chemical composition of the dehydrated sweet potatoes and corn-soybean silage used in both trials is given in table 5. The percentage of crude protein for

TABLE 4

*Feed consumption (air-dry basis) of each group on silage and dehydrated sweet potatoes during each period of trial 2*

Feed	Period (20 d.)			Av.
	1	2	3	
	(lb.)	(lb.)	(lb.)	(lb.)
Dehydrated sweet potatoes	1,562	1,451	1,580	1,531
Silage	1,620	1,597	1,586	1,601

the silage in the 1949-1950 trial is 9.75, as compared to 5.77 in the 1948-1949 trial. This higher percentage is due to the greater proportion of soybeans in the silage.

## DISCUSSION

In both trials, the amount of 4 per cent F.C.M. produced was similar whether the animals were consuming silage or dehydrated sweet potatoes. The groups

TABLE 5

*Chemical composition of dehydrated sweet potatoes and corn-soybean silage (dry matter basis)*

Feed	Dry matter	Crude protein	Crude fat	Nitrogen- free extract	Crude fiber	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
1948-49						
Dehydrated sweet potatoes <sup>a</sup>	91.34	5.47	0.38	86.80	4.13	3.21
Dehydrated sweet potatoes <sup>b</sup>	90.35	5.88	0.47	85.45	4.23	3.96
Silage	28.1	5.77	1.21	51.14	33.92	7.96
1949-50						
Dehydrated sweet potatoes <sup>c</sup>	90.1	5.22	0.55	86.57	4.55	3.11
Silage	28.6	9.75	2.55	54.98	27.45	5.27

<sup>a</sup> Standard

<sup>b</sup> Weevily

<sup>c</sup> Mixture of a and b

also consumed approximately the same amount of these feeds on the air-dry basis. The average milk productions per cow per day for both trials when fed corn-soybean silage or dehydrated sweet potatoes were 23.3 and 23.7 lb., respectively.

No evidence of digestive disturbances was observed in any of the animals. Since some of the animals refused part or all of the dehydrated sweet potatoes when this product completely replaced silage, it is recommended that sweet potatoes should be substituted gradually when changing feed.

The cost of the silage was estimated at approximately \$10 per ton of fresh material or \$1.50 per 100 lb. of air-dry silage, while the dehydrated sweet potatoes cost approximately \$3 per 100 lb. on the market. Under existing levels of production and dehydration costs, dehydrated sweet potatoes are uneconomical to use as a substitute for silage in maintaining milk production at a normal level except during the periods when no silage or pasture is available. The cost of using fresh sweet potatoes is much lower than that of dehydrated potatoes but this product cannot be kept for any length of time unless properly protected against cold or dehydration. One pound of fresh sweet potatoes is approximately equivalent to 1 lb. of fresh silage for milk production, since these products both contain approximately 70 per cent moisture. The dehydrated sweet potatoes were fed wet so as to simulate silage in bulk. Sweet potatoes appear to have a stimulating effect on milk production similar to that of corn-soybean silage.

#### SUMMARY

Two feeding trials were conducted on substituting dehydrated sweet potatoes for corn-soybean silage during the winter months of 1948-1949 and 1949-1950.

The cows produced the same amount of 4 per cent fat-corrected milk on the dehydrated sweet potatoes as when they were on the silage, the averages for both trials being 23.3 and 23.7 lb. per cow per day, respectively. Apparently dehydrated sweet potatoes may serve as a good replacement for silage for milk production, especially when pastures are poor and silage unavailable.

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# THE FERTILITY OF BOVINE SEMEN IN CITRATE-YOLK EXTENDERS CONTAINING ADDED CATALASE

N. L. VANDEMARK

*Department of Dairy Science, University of Illinois, Urbana*

AND

R. W. BRATTON AND R. H. FOOTE

*Laboratory of Animal Breeding and Artificial Insemination*

*Department of Animal Husbandry, Cornell University, Ithaca, N. Y.*

Investigations by VanDemark *et al.* (12) have shown that high oxygen tensions are detrimental to the livability of bovine spermatozoa in the 3.6 citrate-yolk extender or diluter. Presumably, the decreased livability reflects an increased  $\text{H}_2\text{O}_2$  production, as has been demonstrated by Tosic and Walton (11) and Tosic (10). The recent report by Prince and Almquist (5) that agitation of semen in partially filled tubes was harmful to spermatozoan survival also suggests the possibility of oxygen damage due to excessive aeration.

Evans (3) has shown that the fertilizing capacity of *Arbacia* spermatozoa was reduced by treatment with  $\text{H}_2\text{O}_2$ . Retardation of the cleavage time in the fertilized ova resulted. Wyss *et al.* (13) have shown that the mutation rate of *Staphylococcus aureus* increased when the cultures were exposed to  $\text{H}_2\text{O}_2$  in the media. These effects of  $\text{H}_2\text{O}_2$  were negated by the addition of catalase to the media.

In view of the earlier work on the improvement in the livability of bovine spermatozoa from additions of catalase, the effects of  $\text{H}_2\text{O}_2$  on *Arbacia* spermatozoa and certain bacteria, it seems reasonable to postulate that catalase might, through the same process, improve the fertility of bovine spermatozoa used in artificial breeding.

No known reports have been made indicating the relationship between the livability of bovine spermatozoa under conditions of high oxygen tension and their fertility. Similarly, the fertility of bovine spermatozoa in extenders or diluters containing added catalase has not been reported. The results reported by Prince and Almquist (5) and by VanDemark *et al.* (12) were obtained when using 3.6 citrate-yolk extender. The experiment reported herein was designed to compare the fertility of bovine spermatozoa in citrate-yolk and citrate-sulfanilamide-yolk extenders with that of spermatozoa stored in these same extenders but containing added catalase.

## EXPERIMENTAL PROCEDURE

The experimental design was a  $4 \times 4$  Latin square consisting of four experimental extenders, four groups of technician-inseminators and four insemination periods. The insemination periods represented the semen shipped during a 4-day period.

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The compositions of the buffers and extenders are shown in table 1. The buffers were prepared once each week and the extenders were prepared during the afternoon of the day before they were to be used. Catalase was added at the time the extenders were prepared at a rate of one part of Vitazyme Catalase Sarrett to 10,000 parts of extender. This resulted in a lower catalase concentration than was used by VanDemark *et al.* (12), but this concentration was still several times that needed to eliminate the  $H_2O_2$  as fast as it was produced (11), if there were no interfering substances.

TABLE 1  
*Composition of buffers and extenders*

	3.6 CY <sup>a</sup> without added catalase	3.6 CY with added catalase	3.6 CSAY <sup>b</sup> without added catalase	3.6 CSAY with added catalase
Buffer:				
$Na_2C_6H_5O_7 \cdot 2H_2O$ (g.)	36.0	36.0	36.0	36.0
Sulfanilamide (g.)			6.0	6.0
Water (redistilled over glass) to final vol. (ml.)	1000.	1000.	1000.	1000.
Extender:				
Ratio of egg yolk to buffer	1:1	1:1	1:1	1:1
Ratio of added catalase to extender <sup>c</sup>		1:10,000		1:10,000

<sup>a, b</sup> 3.6 indicates the percentage of citrate in the buffers. C=citrate; 8A=sulfanilamide; Y=egg yolk.

<sup>c</sup> The catalase preparation used contained approximately 700–800 units of catalase/ml. (10). (10).

Semen for these studies was obtained from Holstein bulls in the active stud of the New York Artificial Breeders' Cooperative, Inc., and consisted of those ejaculates which contained  $500 \times 10^6$  or more spermatozoa per milliliter, of which 50 per cent or more were motile, as determined by routine procedures (1, 6).

Immediately after collection, the semen was extended at a rate of approximately 1 to 4 in 3.6 citrate-yolk without added catalase and cooled according to the procedure of Foote and Bratton (4). Final extension to a standard number of motile spermatozoa (approximately  $10 \times 10^6$  per milliliter of extender) was made at a temperature of approximately 5° C. with the partially extended semen sample and the final extenders at these same temperatures.

An 8-ml. portion from each of the extended semen samples was stored at 5° C. During storage, each sample was mixed and portions withdrawn from it to simulate the field practice of handling semen at the time the technician performs an insemination. During the first day of storage, the samples were mixed five times but no semen was withdrawn. On the second day they were mixed seven times and three portions of 2 ml. each were removed at 2-hr. intervals. Estimations of the per cent of progressively motile spermatozoa were made microscopically at 3, 24, 48 and 72 hr. of storage and used as a basis for comparing the livability of the spermatozoa during storage.

Fertility was estimated from the 60- to 90-day non-returns to first and second service cows and expressed as per cent non-returns.

#### RESULTS AND DISCUSSION

Forty-seven ejaculates from 24 bulls were used for insemination. The average number of motile spermatozoa per milliliter of extended semen was  $11.9 \times 10^6$ . Table 2 gives the estimated average percentages of motile spermatozoa in the experimental extenders after 3, 24, 48 and 72 hr. of storage at 5° C. The differences between spermatozoan livability in the different extenders were not significant. These results are in contrast to those previously reported by VanDemark *et al.* (12) for semen extended in 3.6 citrate-yolk with and without catalase and mixed at regular intervals.

TABLE 2  
*Livability of bull spermatozoa during storage at 5° C. in extenders with and without added catalase*

Duration of storage <sup>a</sup>  (hr.)	3.6 CY <sup>a</sup> without added catalase	% motile spermatozoa		
		3.6 CY with added catalase	3.6 CSAY <sup>b</sup> without added catalase	3.6 CSAY with added catalase
3	66	66	66	67
24	62	64	63	64
48	58	58	58	57
72	53	55	52	53

a, b See footnotes for table 1.

Table 3 gives the number of first, second and the combined number of first and second service cows inseminated and the mean per cent 60- to 90-day non-returns for these groups of cows. The means for the per cent non-returns to both first service cows and second service cows show that the fertility level of the semen with added catalase varied but little from the level of the semen without added catalase. On the basis of the combined first and second service cows, the average per cent non-returns for extenders containing catalase was 61.2 and for those not containing catalase, 61.8.

Since no improvement from added catalase was shown in either spermatozoan livability or fertility in the present study, it is possible that the  $H_2O_2$  level of the extended semen during the 2 days in which the majority of inseminations were being made was not high enough to be detrimental. This may have been a consequence, in part, of the procedure used in cooling the semen. In the studies by VanDemark *et al.* (12) in which oxygen damage to spermatozoa was alleviated by catalase, the semen was gradually cooled to 5° C. and then extended with cold (5° C.) citrate-yolk containing added catalase. Since that time Foote and Bratton (4) have shown an improvement in fertility from partially extending the semen with the citrate-yolk before cooling. This cooling procedure is in routine use at the New York Artificial Breeders' Cooperative and was used in this experiment.



The change in cooling procedure also may have been responsible for the small difference in the fertility level shown between semen extended with citrate-yolk and that extended with citrate-sulfanilamide-yolk. Earlier investigations (2, 7, 8), in which the fertility of semen in extenders with and without added sulfanilamide was compared, showed an increase of approximately five percentage units in 60- to 90-day non-returns to first service cows in favor of the samples with added sulfanilamide.

#### SUMMARY

Using the split sample technique, the spermatozoan livability and the fertility of 47 semen samples from 24 Holstein bulls were studied when extended to contain approximately  $11.9 \times 10^6$  motile spermatozoa per milliliter in citrate-yolk and citrate-sulfanilamide-yolk extenders with and without added catalase.

TABLE 3

*Fertility level of bull semen extended with and without added catalase.  
(Based on 60- to 90-day non-returns to 1st and 2nd service cows)*

	3.6 CY <sup>a</sup> without added catalase	3.6 CY with added catalase	3.6 CSA Y <sup>b</sup> without added catalase	3.6 CSA Y with added catalase
Total number of: 1st service cows	1205	1168	1172	1140
60- to 90-day non- returns (mean %) .	62.0	60.8	63.0	63.1
2nd service cows	575	554	528	569
60- to 90-day non- returns (mean %) .	61.0	58.7	57.9	57.3
Combined 1st and 2nd service cows	1780	1722	1700	1709
60- to 90-day non- returns (mean %)	61.4	60.7	62.2	61.7

<sup>a, b</sup> See footnotes for table 1.

On the basis of the average per cent 60- to 90-day non-returns to service to approximately 1,700 first and second service cows per treatment, the extenders compared as follows: 3.6 citrate-yolk without added catalase, 61.4; 3.6 citrate-yolk with added catalase, 60.7; 3.6 citrate-sulfanilamide-yolk without added catalase, 62.2; and 3.6 citrate-sulfanilamide-yolk with added catalase, 61.7.

Differences in spermatozoan livability and fertility in the various extenders were not significant.

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## PAROTID GLAND LESIONS IN EXPERIMENTAL BOVINE VITAMIN A DEFICIENCY

E. L. JUNGHERR, C. F. HELMBOLDT AND H. D. EATON

*Departments of Animal Diseases and Animal Industries,  
Storrs Agricultural Experiment Station, University of Connecticut, Storrs*

The study was concerned with clinical, biochemical and pathologic manifestations of vitamin A deficiency in young dairy bulls which had been maintained on a diet low in carotene but otherwise of sufficient caloric value to permit normal growth. The experiments were terminated after an average period of 105 days, when convulsive symptoms were well established. The aim was to elucidate the basic lesions in rapidly developing, uncomplicated A-hypovitaminosis.

The pathology of bovine vitamin A deficiency of different intensity and duration has been studied by a number of workers, but the reports vary with respect to the significance and specificity of the lesions observed.

Ocular changes simulating infectious keratitis were found frequently by Hart and Guilbert (9) under natural conditions ascribed (10) to pinching of the optic nerve by a sphenoidal stenosis. Blindness without observable lesions was shown by Wetzel and Moore (22) to be due to edema of the optic papilla, resulting from increased cerebrospinal fluid pressure, according to Moore and Sykes (18).

The seminiferous tubules of young bulls were found, by Guilbert and Hart (7), to exhibit structural changes, a fact confirmed in detailed studies of Hodgson *et al.* (11), Erb *et al.* (5) and Bratton *et al.* (2).

Nephritic changes interpreted as parenchymatous nephritis were found to be associated with fatally terminating spontaneous cases in the experience of Hart (8). The corresponding experimental lesions were characterized by Langham *et al.* (13) as degenerative in the form of hydropic and necrobiotic alterations in the proximal portions of the nephron and as inflammatory in the form of cellular infiltrations and proliferations in the interstices. There was occasional metaplasia with rare hyperkeratinization of the transitional epithelium of the minor calices and the ureters. In a similar study Thorp *et al.* (21) confirmed these findings and reported only 2 of 25 animals as showing metaplasia in the calices.

The pituitary has been found to present cystic degeneration by Moore (16) or increased fluid between the anterior and posterior lobe by Sutton *et al.* (20). The latter authors also found an increase of "alpha" cells (acidophils) and believed the change to be similar to that in A-deficient rats, although their original studies on this species (19) showed an increase of "beta" cells (basophils). The cellular changes are interpreted as compensatory to the testicular degeneration, paralleling the so-called castration effect. On the basis of 10,000 slaughter-

ing house specimens, Madsen *et al.* (15) considered cystic pituitaries in young cattle as a pathologic expression of A deficiency.

Anasarca or edema of subcutis and adjacent musculature has been described by Creech and Seibold (3) and was used as a criterion by Madsen and Earle (14) in characterizing "old corn" disease as vitamin A deficiency.

Pneumonic lesions were frequently observed by Hart (8) in natural, fatal cases and occasionally under experimental conditions by Thorp *et al.* (21). The latter authors also reported mild hyperplastic lesions in the small intestine and necrobiotic changes of like intensity in the liver.

On the whole, it may be seen that the now universally recognized basic lesion of A-hypovitaminosis, namely squamous metaplasia with varying degrees of hyperkeratinization (23), has been reported in the kidney only and even there as a distinctly minor alteration.

TABLE 1  
*Age, hemoglobin and carotene and vitamin A liver storage of experimental animals*

			Age in days			Hemoglobin (g./100 ml.)		Final liver storage ( $\gamma$ /g.)	
	No.	Breed	Start	Finish	Difference	Start	Finish	Carotene	Vitamin
First expt.	1	Jersey	339	403	64	11.5	11.5	0.7	0.2
6-15-48	2	Guernsey	53	134	81	8.5	8.4	0.1	0.0
to	3	Guernsey	210	312	102	8.5	10.5	0.3	0.1
9-25-48		Av.	201	283	82	9.5	10.1	0.4	0.1
	4	Control Guernsey	208	310	102	9.4	10.7	0.4	25.7
Second expt.	5	Guernsey <sup>a</sup>	240	375	135	10.0	11.4	0.8	8.2
2-15-49	6	Ayrshire	139	274	135	9.8	10.9	0.4	3.4
to	7	Holstein	148	231	183	10.4	10.1	0.4	0.1
6-30-49		Av.	176	293	118	10.1	10.8	0.5	3.9
	8	Control Holstein	171	306	135	9.9	10.4	0.6	103.0
		Av. Deficient	188	288	100	9.7	10.7	0.5	2.0
		Av. Controls	189	308	119	9.7	10.6	0.5	64.4
		Av. Totals	189	293	105				

<sup>a</sup> Freemartin.

In a recent comprehensive treatise on the pathology of nutritional diseases, Follis (6) emphasized the importance of differentiating between specific and nonspecific damage due to deficiency of a single nutrient.

#### MATERIALS AND METHODS

The experiments were conducted on two groups of four bull calves each, except for one freemartin, representing four standard dairy breeds (one Jersey, four Guernseys, one Ayrshire, two Holsteins). In average terms, the first group, aged 204 days, was treated for 92 days during the summer of 1948, and the second group, aged 173 days, was treated for 126 days during the spring of 1949. The details are presented in table 1.

During treatment, each animal received a daily allowance of 4 lb. of grain mixture<sup>1</sup> containing less than 350  $\mu$ g of carotene per lb. and beet pulp *ad libitum*. One control calf in each group received a daily supplement of 100,000 I.U. vitamin A from dogfish oil containing 25 per cent crude soybean lecithin. Clinical observations were made daily, and hemoglobin, plasma carotene and vitamin A content were determined weekly. Spinal fluid pressure readings according to Moore (17) and liver biopsies for histologic study were obtained approximately once per month. All of the animals were sacrificed when they showed daily convulsions, except for one which died on the 64th experimental day. The livers were frozen for later carotene and vitamin A determinations and the tissues subjected to thorough gross and microscopic examination.

#### CLINICOPATHOLOGIC RESULTS

Symptoms of spasmodic convulsions became manifest in the animal which later died, after about 45 days on experiment, in the others after about 75 days. The spasms increased in frequency until they occurred three to four times every day and were accentuated by sexual excitement. Bloat and diarrhea occurred occasionally. Some animals manifested impaired eyesight and exophthalmus.

The average clinicopathologic data for six treated and two control animals were as follows:

**Hemoglobin.** Expressed in grams per 100 ml. both the treated and control groups averaged 9.7 at the beginning of the experiment and 10.7 *versus* 10.6 at the end. There was no significant difference between groups, but all of the hemoglobin values increased slightly during the course of the experiments. The details are presented in table 1.

**Spinal fluid pressure.** Expressed in millimeters of water, the average values obtained in the first experiment were 307 in the treated group as against 322 in the control group at the start and 260 in the treated against 100 in the control group at the end. Later experiences showed that these values probably had been exaggerated by excitement. In the second experiment, the measurements averaged 107 for the treated against 91 for the control groups at the start and 190 *versus* 155 at the end.

The total averages were 207 for both the treated and control groups at the start and 225 for the treated *versus* 128 for the control groups at the end. There was a relative increase in spinal fluid pressure in the deficient group as compared with the control group, in accordance with the literature (18).

**Liver biopsies.** Narrow cylinders of hepatic tissue, obtained with an instrument designed for human prostatic biopsy, were fixed in Zenker's fluid, formol-saline and absolute alcohol, respectively. Special strains were applied to bring out cellular detail, neutral fat and glycogen. In general, both the deficient and the control groups failed to show any uniform structural changes or fatty meta-

<sup>1</sup> Grain mixture: Ground barley, 419.5; crimped oats, 500; wheat bran, 500; linseed oil meal (solvent process), 150; soybean oil meal (solvent process), 150; molasses, 200; 500-potency B-Y dried fermentation solubles, 40; steamed bone meal, 20; salt, 20; irradiated yeast (Standard Brand, type 9-F, 9,000 I.U. vitamin D per gram), 0.5; total, 2,000 lb.

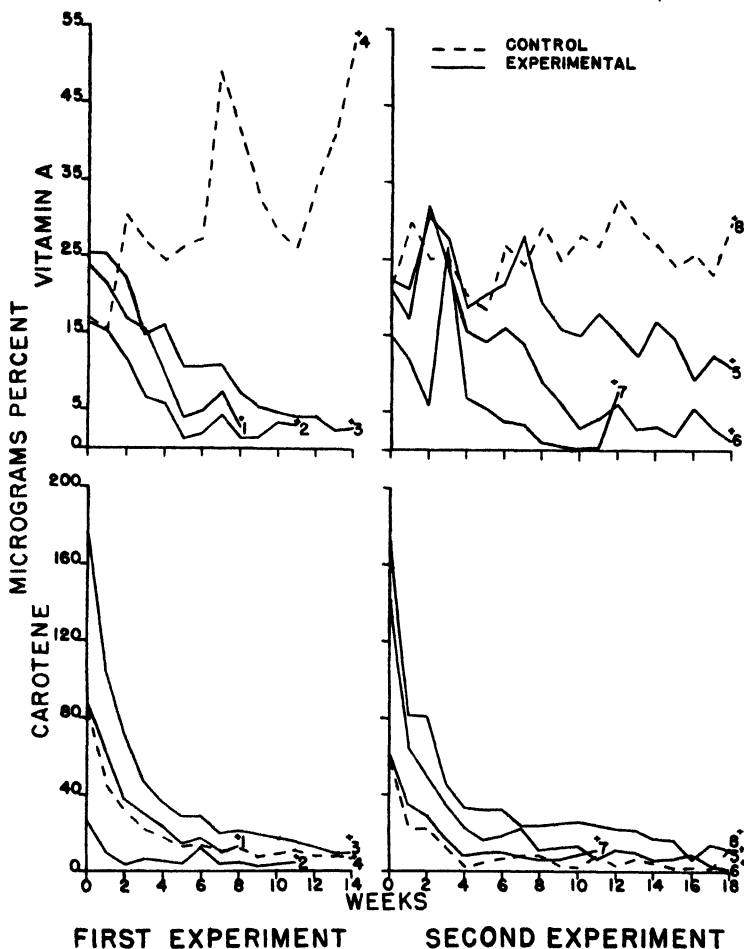
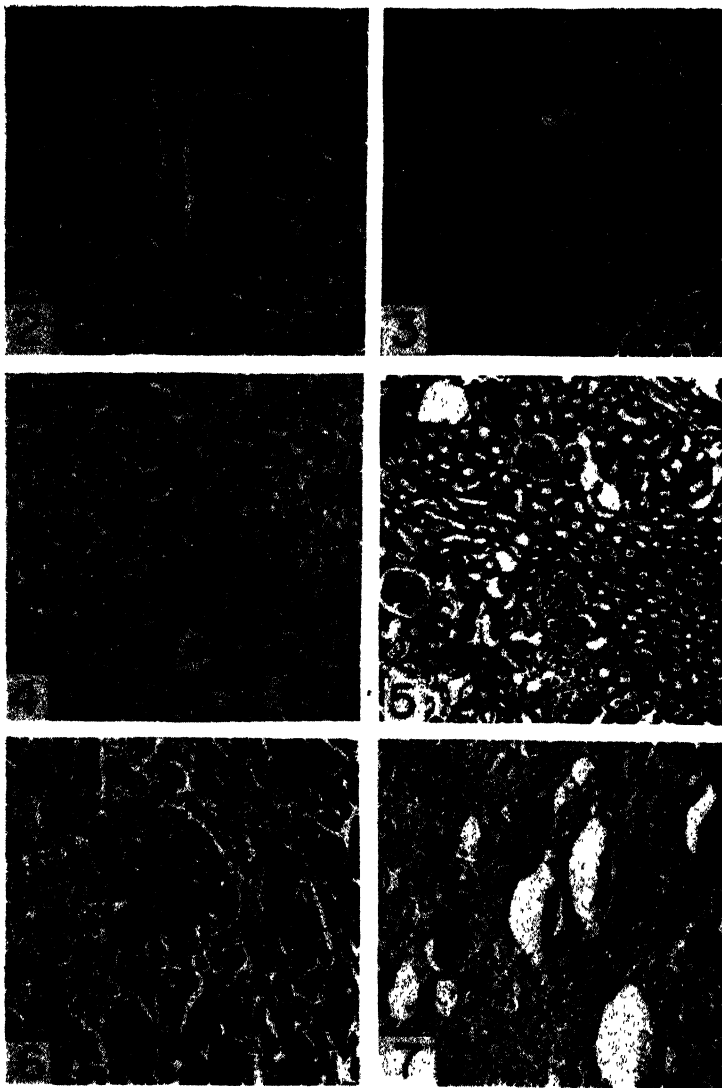


FIG. 1. Blood plasma carotene and vitamin A values obtained by weekly determinations during the course of the first and second experiment. The numbers at the end of each line refer to the no. of the animal as listed in table 1.

morphosis. There was fair-to-good glycogen storage throughout the course of the experiments.

**Blood plasma carotene.** Expressed in micrograms per 100 ml. the treated groups averaged 184 and the controls 132 at the beginning and 8 versus 9 at the end. Thus, there was no significant difference between groups. Both the deficient and the supplemented animals showed an approximately equal regression of plasma carotene under the conditions of these experiments. The details are presented in figure 1.

**Blood plasma vitamin A.** Expressed like carotene, the treated groups averaged 22.6 and the controls 22 at the start and 3.6 versus 42.5 at the end. Thus,



the deficient animals exhibited a marked decrease and the supplemented animals a corresponding increase in plasma vitamin A levels (fig. 1).

*Final liver storage.* Expressed in micrograms per gram of liver, carotene in both the treated and control groups averaged 0.5 thereby failing to show differences due to treatment, in line with the corresponding plasma values.

Vitamin A, on the other hand, averaged 2.0 in the treated group, as against 64.4 in the control group. There was, therefore, a significantly higher storage in the supplemented groups in comparison with the deficient ones, as was to be

expected from the corresponding plasma values. The details are presented in table 1.

#### **PATHOLOGIC RESULTS**

On gross examination, animal no. 1 (table 1) which died presented significant hepatic changes in the form of multiple poppy-seed sized yellowish areas, which were interpreted as focal necrosis.

Histopathologically, animal no. 2 showed focal necrosis (fig. 2), portal cirrhosis (fig. 3) of the liver and early exudative pneumonia (fig. 4). Animal no. 6 showed focal interstitial nephritis. Brain sections often showed perivascular and perineuronal edema, so-called lamina cribrosa. Because of their irregular occurrence, these lesions were considered as due to mild intercurrent diseases, not necessarily associated with treatment.

Microscopic changes of probable significance were found in the pituitary and the thyroid. The pituitary, especially the anterior lobe, has been stated in the literature to show both cystic (15) and cellular changes (20). In the present material, microcysts were found in both the treated and the control groups and, therefore, not accorded significance. The differential cellular picture, as presented in Bouin's fixed Masson's trichrome preparations, showed in the controls massive ribbon-like accumulations of acidophils in the periphery, leaving a narrow central area composed primarily of chromophobes and basophils (fig. 6). The principal differences in the treated animals were an apparent reduction of both chromatic cellular elements and a consequent predominance of chromophobes. In some sections from affected animals it was impossible to demonstrate any appreciable number of basophils (fig. 7). Although these numerical differences were based on estimates and not differential counts, they were contrary to expectations from the literature (19, 20) and suggested that this subject requires reinvestigation.

The thyroid of treated animals showed mild hyperplasia, while control animals presented more or less uniformly sized and well filled follicles lined by low cuboidal epithelium (fig. 8). Treated animals exhibited many small follicles with high cuboidal or nearly columnar epithelium which had a tendency to encroach upon the lumen (fig. 9). Other follicles varied widely in size and contained colloid with markedly scalloped margins. Hyperplasia of the thyroid

**FIG. 2.** Liver of no. 2. Peripheral necrosis—Karyolysis of liver cells around interlobular vein.

**FIG. 3.** Liver of no. 2. Portal cirrhosis—Marked increase of connective tissues in portal island accompanied by proliferation of bile ducts.

**FIG. 4.** Lung of no. 2. Exudative pneumonia—Polynuclear and mononuclear cells in alveoli, bronchiole (low center) and alveolar ducts (high center).

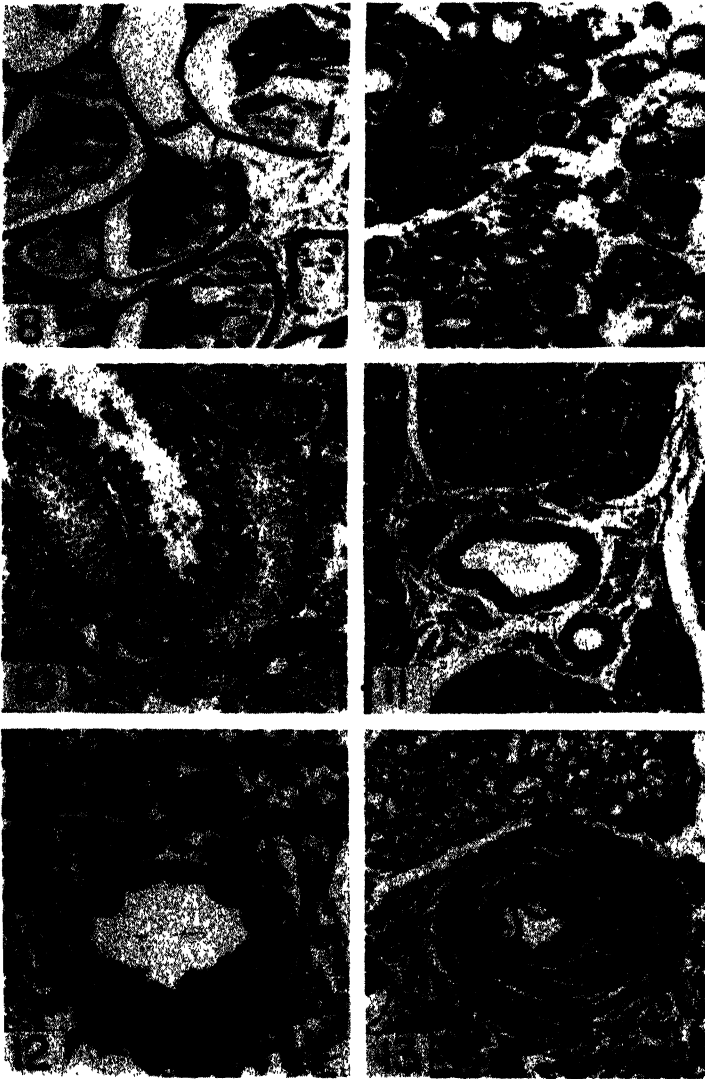
**FIG. 5.** Kidney of no. 6. Interstitial nephritis—An atrophied glomerulus in low center surrounded by interstitial round cell infiltration.

**FIG. 6.** Anterior pituitary of no. 8. Normal—Massive cords of acidophiles (dark) separated by narrow cords of basophiles and chromophobes (light).

**FIG. 7.** Anterior pituitary of no. 5. Vitamin A deficiency—Broad bands of chromophobes (light) and islands of acidophiles (dark). Many microcysts.

All figures are photomicrographs of paraffin sections stained with hematoxylin-triosin, 80 x. The numbers refer to the experimental animals listed in table 1.





in vitamin A deficiency may be compensatory to increased stress upon this organ, which is known to have an important function in the conversion of carotene to vitamin A (4).

Specific microscopic changes were observed in the testes and the parotid gland. The testicular changes, which have been reported frequently in the literature (2, 5, 7, 11), consisted in the present material of various degrees of retardation in spermatogenesis. In the most advanced cases, the seminiferous epithelium in certain tubules was extremely cell-poor, with only a few Sertoli

cells near the basement membrane. In most instances spermatogenesis had not progressed beyond the spermatogonial stage. There were only isolated primary and secondary spermatocytes, but there was no evidence of any orderly progressive maturation. However, the abnormalities often were confined to certain selected tubuli with adjacent ones appearing almost normal (fig. 10).

The parotid gland, which, as far as the authors are aware, has not been mentioned in the literature, proved to be the only organ that regularly showed pathognomonic changes of vitamin A deficiency.

The parotid as the largest, chiefly serous salivary gland has a complex duct system which terminates in the oral cavity (Stenson's duct) opposite the second upper molar. The serous alveoli drain into prominent intralobular and intercalated ducts which are lined by a single layer of columnar cells with centrally located nuclei. Where the ducts reach the interlobular connective tissue septa, the epithelium changes to a pseudostratified columnar epithelium with the nuclei in two or more layers (fig. 11) and maintains this architecture to its termination (1).

In vitamin A deficiency the specific changes were confined to the interlobular ducts of both small and large diameter. There the normally columnar epithelium in some of these ducts had changed to squamous epithelium (fig. 12) accompanied occasionally by hyperkeratinization. The pathologic epithelium was markedly hyperplastic and built up in irregular layers. The germinal layers were relatively rich in mitotic figures with the cytoplasm of some hypertrophied prickle cells occasionally containing round bodies, suggestive of dyskeratotic degeneration. The innermost surface cells not infrequently formed loops or bridges over vacuolar spaces (fig. 13) presumably containing retained secretion. Cross sections of affected interlobular ducts showed the narrowing effect of the pathologic process on the ductal patency and obviously suggested a pathogenetic relationship between stenosis of the parotid duct and vitamin A deficiency. On the whole, the lesions reflected the squamous metaplasia considered to be the basic lesion of vitamin A deficiency in mammals and birds.

Apparently the parotid gland in the bovine is one of the organs of predilec-

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FIG. 8. Thyroid gland of no. 4. Normal—Large, moderately filled follicles, lined by low cuboidal epithelium. Slightly hypoplastic state.

FIG. 9. Thyroid gland of no. 7. Vitamin A deficiency—Small follicles lined by high cuboidal to columnar epithelium, sometimes obliterating lumen. Colloid has scalloped margins and stains deeply. Hyperplastic state.

FIG. 10. Testis of no. 1. Vitamin A deficiency—Cessation of spermatogenesis in two lateral seminiferous tubules (uniformly gray), other tubules normal.

FIG. 11. Parotid gland of 6-week-old calf affected with pulmonary abscesses, caused by *Spherophorus necrophorus*. Normal—Peripheral alveolar tissue; central H-like interlobular connective tissue with normal interlobular ducts lined by two-layered pseudostratified columnar epithelium.

FIG. 12. Parotid gland of no. 1. Vitamin A deficiency—Interlobular connective tissue with large interlobular duct lined by irregularly built-up metaplastic squamous epithelium. Stratum corneum is nucleated (parakeratotic).

FIG. 13. Parotid gland of no. 7. Vitamin A deficiency—Alveolar tissue in upper third. Large interlobular duct in center with thickened wall and advanced squamous metaplasia of lining epithelium showing interepithelial bridges and microcysts.

tion for exhibiting specific lesions of vitamin A deficiency, in distinction from chickens where the corresponding locus seems to be in the mucocutaneous junction of the nasal septum (12).

#### SUMMARY

Two groups of four dairy bull calves, averaging 189 days in age were fed a grain mixture containing less than 350  $\gamma$  per lb. of carotene and beet pulp *ad libitum* for about 105 days. One control calf in each group received a daily supplement of 100,000 I.U. of vitamin A from dogfish oil containing 25 per cent crude soybean lecithin. In weekly determinations, the hemaglobin values showed no significant changes for treated and supplemented animals, plasma carotene regressed in both groups, while plasma vitamin A was markedly higher in the supplemented animals. The same relationship was reflected in the final liver storage of carotene and vitamin A. Monthly readings of spinal fluid pressure indicated a relative rise in the treated animals, while simultaneous liver biopsies failed to manifest changes in glycogen and fat storage.

Pathologic studies of the animals killed after convulsions occurred daily failed to show consistent gross lesions. Irregularly occurring microscopic lesions of focal necrosis and/or cirrhosis in the liver, pneumonia and mild interstitial nephritis suggested intercurrent diseases. Consistent changes were found in the anterior pituitary showing a decrease in the chromatic cells and in the thyroid showing mild hyperplasia. The testes manifested retarded spermatogenesis in some seminiferous tubuli. The parotid gland showed a high incidence of specific squamous metaplasia in the interlobular ducts.

The parotid gland appears to be especially prone to exhibit specific histopathologic alterations of A-hypovitaminosis and is the only organ so far ascertained that lends itself to specific morphologic diagnosis of vitamin A deficiency in the ox.

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## SELECTION OF SAMPLE IN DETERMINATION OF THE STREPTOCOCCAL FLORA OF THE UDDER<sup>1</sup>

E. M. KESLER, J. J. REID AND C. B. KNODT  
*The Pennsylvania Agricultural Experiment Station*

The selection of samples of milk for study of the udder bacterial flora has been a subject of controversy (4). Several investigators have observed the composition of milk obtained at various stages of milking (5) but few have reported on the relative usefulness of such samples in the determination of the udder flora. Murphy (6) compared four successively drawn 10-ml. quantities with the strippings and reported that, in general, the same flora appeared throughout. Numbers of microorganisms were found, however, to decrease progressively with successive samples and the leucocyte count likewise decreased. Cunningham *et al.* (2) arrived at the conclusion that strict foremilk should be used in a determination of udder flora, as it contained essentially the same flora and in larger quantities than samples from a later stage of milking. Bull *et al.* (1), however, observed that the milk in the teat canal contained a wider variety of organisms than did milk from the udder. Little (3) in a study of an animal inoculated with a hemolytic streptococcus, found that mid-milk and strippings showed the presence of relatively few streptococci in comparison with the large numbers found in strict foremilk. Little and Plastringe (4) have suggested that in experimental work the strict foremilk be used, while in routine examinations 5 ml. may be discarded before sampling.

The rather scant and conflicting data on the selection of a sample for the determination of the udder flora led the authors to the conclusion that further study of the sampling procedure was necessary. Inasmuch as the authors were interested primarily in the streptococcal flora of the udder at the time this study was conducted, this report was limited to the choice of a sample for the determination of streptococci present.

### EXPERIMENTAL

Forty-seven animals in the College herds were chosen for the purpose of this study. Various breeds, ages, stages of lactation and levels of production were represented. Five animals were suffering from chronic mastitis and several others had past histories of udder trouble. Many of the animals were termed normal on the basis of periodic laboratory examination of the milk and no history of clinical mastitis.

From these animals, 940 samples of milk were obtained for this study. Prior to sampling, the udder and flanks of each animal were washed with a fresh solution of hypochlorite containing approximately 200 ppm. available

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chlorine. Special care was taken to insure that the ends of the teats were well cleansed. Samples then were drawn into sterile bottles containing 0.2 ml. of a sterile solution of the following composition: 0.2 g. brilliant green, 0.75 g. sodium azide, 10.0 g. glucose and 200.0 ml. distilled water. This solution had been filtered and then sterilized in the autoclave at 15 lb. pressure for 20 min. and had been placed aseptically in the previously sterilized bottles.

Five 15-ml. samples were obtained from each quarter and labeled A to E, inclusive. Sample A comprised the strict foremilk, B and C represented, respectively, the second and third 15-ml. portions of milk drawn from the quarter. The machine then was placed on the animal and, in the judgment of the operator, removed in order to obtain the fourth sample, D, at the time one-half the milk had been removed from the udder. Sample E was obtained following the final removal of the machine from the animal and represented strippings.

The samples were incubated for a period of 16 hr. at 37° C., following which microscopic examinations were made by means of a modification of the standard direct count. Using a loop delivering 0.01 ml. and a surface area of 2 cm.<sup>2</sup>, smears were made which were air dried, defatted with xylol and stained

TABLE 1

*Comparison of flora and leucocyte content of samples of milk obtained at various stages of milking*

	Number of positive samples among the 188 of each group examined				
	A Foremilk	B 2d 15 ml.	C 3d 15 ml.	D Mid-milk	E Strippings
Long chain streptococci	17	16	17	11	8
<i>Streptococcus agalactiae</i>	7	7	6	5	5
Beta hemolytic colonies	27	30	32	23	24
Leucocyte content of more than 1,000,000/ml.	41	43	42	53	66

with the Newman-Lampert formula II. Actual counts of bacteria were not made but the following information was obtained: leucocyte count, relative numbers of long chain streptococci, medium chain streptococci, short chain streptococci, rods and staphylococcal clusters.

From all samples streaks were made on Edward's medium (4). These plates were incubated for a period of 48 hr. at 37° C. and examined for nature of growth, if present. Further examinations of isolates from these plates were made in those cases in which it proved necessary to determine the nature of the streptococci present.

#### RESULTS

Examination of the data obtained reveals certain pronounced differences in bacterial flora and leucocyte content in the several samples drawn from a quarter. The significant differences found are presented in table 1. Of particular significance is the progressive decrease in the number of samples showing long chain streptococci and the increase in samples containing more than 1,000,000 leucocytes per milliliter as successive samples are obtained in the milking. It

also would appear that the presence of *Streptococcus agalactiae* is more likely to be observed in foremilk than in later sampling.

In table 2 is presented an approximation of the relative numbers of long chain streptococci noted in the positive samples and of leucocytes in those samples containing more than 1,000,000 per milliliter. It will be noted that not only do the number of samples showing long chain streptococci decrease in successive sampling (table 1), but there is a similar decrease in the number of chains noted

TABLE 2

*Relative numbers of long chain streptococci and leucocytes present in the positive samples shown in table 1*

	Relative numbers in positive samples				
	A Foremilk	B 2d 15 ml.	C 3d 15 ml.	D Mid-milk	E Strippings
Chains of long chain streptococci	26	23	18	2	1
Leucocytes, as millions/ml.	2.8	2.9	3.4	4.1	6.4

in microscopic examination of the incubated milk. Likewise, the increase in the number of quarters showing excessive leucocytes (table 1) is accompanied by progressive increases in numbers per milliliter in the questionable samples.

In table 3 are presented detailed data obtained in the study of the strict foremilk (A) and the second 15 ml. (B) drawn. The data suggest little actual difference in the two series.

TABLE 3

*Comparison of microflora and incidence of excessive leucocytes in strict foremilk and the following 15-ml. sample*

Incidence of	Numbers of positive samples among the 188 of each group examined	
	A Foremilk	B 2d 15 ml.
Micrococci in smears	157	158
Pairs of cocci in smears	130	136
Short chain streptococci in smears	99	104
Medium chain streptococci in smears	24	19
Long chain streptococci in smears	17	16
Staphylococci in smears	10	12
Beta hemolysis on plates	27	30
Gamma hemolysis on plates	6	5
<i>Streptococcus agalactiae</i> on plates	7	7
Leucocytes in excess of 1,000,000/ml.	41	43

#### SUMMARY

The streptococcal flora and the leucocyte content of the quarters of 47 dairy cows were determined for five different stages of milking. These stages were as follows: first 15-ml. drawn, second 15-ml., third 15-ml., mid-milk and strippings. Little difference was noted between the strict foremilk and the second 15-ml. sample. It would seem that either would be useful in a determination of the streptococcal flora. On the other hand, not all quarters found shedding *Str.*

*agalactiae* by the use of these samples would have been detected had either mid-milk or strippings been used as the basis of the tests. The presence of long chain streptococci other than *Str. agalactiae* likewise would have been missed. Excessive numbers of leucocytes appeared more often and in larger numbers in the later samples.

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# THE METHYL KETONES OF BLUE CHEESE AND THEIR RELATION TO ITS FLAVOR<sup>1</sup>

STUART PATTON

*Department of Dairy Husbandry,  
The Pennsylvania Agricultural Experiment Station, State College*

The interest of research workers has been focused upon the origin and nature of mold-ripened cheese flavor for a number of years. According to Currie (1), early investigators attributed the characteristic flavor of the cheese to esters or ketones. From his own extensive work, Currie concluded that the flavor of roquefort cheese is due to the presence of certain fatty acids or their readily hydrolyzable salts. Hammer and Bryant (2) believe that one or more methyl ketones, heptanone-2 in particular, are responsible for part of the characteristic flavor of blue cheese. These workers demonstrated the conversion of *n*-caprylic acid to heptanone-2 in a milk medium inoculated with *Penicillium roqueforti*. The relationship of certain chemical analyses to the flavor of blue cheese has been studied recently by Parmelee and Nelson (4).

The odor of blue cheese strongly suggests the presence of ketones. Evidence from the literature also supports this contention. However, insofar as is known, no ketones have been conclusively identified as constituents of a mold-ripened cheese. The present investigation was conducted to amplify this point.

## EXPERIMENTAL

The blue cheeses used in these experiments were representative of the type produced at the Pennsylvania State College Creamery. They were 6 mo. old, of good saleable quality and averaged approximately 5 lb. in weight.

*Preliminary experiments.* Considerable preliminary experimentation was necessary in order to develop effective methods of recovering the ketones in good yield. In these initial experiments, one cheese constituted the starting material. Several similar trials were made during which it was possible not only to improve the steam distillation method of isolation but to collect a considerable amount of presumptive data relative to the specific ketones present in the distillate from blue cheese. This distillate was observed invariably to give positive results with certain tests for methyl ketones. These tests included the color reaction with nitroprusside reagent, the iodoform reaction and the reaction with semicarbazide or 2,4-dinitrophenylhydrazine reagents to form carbonyl derivatives.

The ketones were removed from the distillate by extracting several times with equal volumes of ethyl ether. The ether solution was dried with anhydrous sodium sulfate and the ketones concentrated by removing the solvent on a warm

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<sup>1</sup> Authorized for publication as paper no. 1597 on May 2, 1950, in the Journal Series of the Pennsylvania Agricultural Experiment Station.

water bath. The solvent-free residue had a very potent aroma of blue cheese. The yield of crude mixed ketones obtained under these conditions was improved somewhat with each experiment but never exceeded 1.5 g. per 5 lb. of cheese. Attempts to separate the ketones by distillation were only partially successful because of the small yields. However, data on the fractions obtained indicated the presence of heptanone-2 and nonanone-2 rather conclusively. These data are omitted for the sake of brevity, since similar data from a large scale experiment are presented hereafter in detail.

The distillate still gave positive nitroprusside and iodoform reactions after extraction with ether. Semicarbazone (melting point,  $190^{\circ}\text{C.}$ ) and 2,4-dinitrophenylhydrazone (melting point,  $125\text{--}126^{\circ}\text{C.}$ ) derivatives were prepared from it. The melting points of these derivatives agreed well with values reported for the same known derivatives of acetone (3). In addition, these two derivatives showed no depression in melting point when admixed with corresponding authentic samples. Thus, the presence of acetone was demonstrated consistently in several trials.

*Procedure for a large-scale experiment.* The principal problem encountered in the preliminary trials concerned insufficient yield of mixed ketones which complicated fractionating of the mixture and obtaining reliable fraction boiling ranges and refractive indices. To overcome these difficulties, the amount of starting material was increased from one to three cheeses (16 lb., 3 oz.). A continuous ether extraction procedure, which also appeared to improve the yield, was substituted for extraction of the distillate in a separatory funnel.

The procedure employed for isolating and concentrating the ketones in this experiment was as follows: Three cheeses were mixed with 5 l. of cold water in a blending bowl until a homogeneous mass, free of lumps, was obtained. This mixture was transferred to an 18-l. pyrex jug. The jug and contents were fitted into a steam distillation apparatus of conventional design. The steam was conducted first through a trap, then through the cheese mixture. Effective condensation of the vapors was accomplished by means of two Allihn condensers connected in tandem. The condensate was conducted through a small quantity of ice water by extending a piece of glass tubing from the end of the condenser to the bottom of the receiving flask. The receiving flask was immersed in crushed ice as an additional precaution against loss of volatile material. The end point of the distillation was determined by the time necessary to exhaust the ketonic odor from the cheese mixture. This required distillation for slightly less than 1 hr. during which time 2 l. of condensate were obtained. The bulk of the odorous material appeared to have distilled within the first 15 to 20 min.

The distillate thus obtained was transferred to a continuous extraction apparatus<sup>2</sup> of 2-l. capacity and extracted for a period of 72 hr. with ethyl ether. This ether had been redistilled several times and rendered free of carbonyl compounds by treatment with 2,4-dinitrophenylhydrazine reagent. The extracted distillate was observed to contain acetone as previously noted. The ether extract was dried and the solvent evaporated as described heretofore. The extract residue (5.2 g.) was transferred to a 25-ml. Erlenmeyer flask from

<sup>2</sup> Ace Glass Co., Vineland, N. J.

which it was fractionally distilled. The fractionating column used in this distillation was a paper-jacketed piece of 10-mm. pyrex tubing containing a 10-in. section packed with  $\frac{1}{8}$ -in. glass helices and fitted with a sidearm delivering from above the column packing to a micro condenser. Boiling ranges were measured with a 360° C., partial immersion thermometer. Refractive indices were determined at 25° C. with a Spencer refractometer. Carbonyl derivatives were prepared from the various fractions according to customary procedures (5), but on a somewhat micro scale.

The data for this experiment are presented in table 1. All fractions reported in the table gave positive iodoform and nitroprusside reactions. They also formed derivatives with 2,4-dinitrophenylhydrazine or semicarbazide.

TABLE 1

*Some properties of fractions obtained by the fractional distillation of material from blue cheese containing a high concentration of methyl ketones*

Fraction no.	Physical properties			Melting points of derivatives		Identity of ketone component
	Major boiling range	Refractive index ( $n_D^{25}$ )	Wt.	2,4-DNPH <sup>a</sup>	Semicarbazone	
	(° C.)		(g.)	(° C.)	(° C.)	
1	78-80	1.3721	0.850	140-141	105-106	Pentanone-2
2	100-111	1.3895	0.340	140-141	105-106	Pentanone-2
3	116-127	1.3992	0.045	<sup>b</sup>	<sup>b</sup>	
4	150-154	1.4075	0.680	72, 90 <sup>c</sup>	121-122	Heptanone-2
5	155-175	1.4075	0.640	55-60	105-110	<sup>d</sup>
6	185-192	1.4160	0.940	38, 55 <sup>c</sup>	118-119	Nonanone-2
7	200-255	1.4290	0.660	38	105-110	<sup>e</sup>
8	non-distilling		0.610			

<sup>a</sup> 2,4-dinitrophenylhydrazine.

<sup>b</sup> Insufficient of the derivatives to permit purification.

<sup>c</sup> Preparations of these derivatives resulted in two forms which had different melting points.

<sup>d</sup> The components of this fraction were a mixture which could not be resolved.

<sup>e</sup> This fraction contained very little methyl ketone.

In order to confirm the identity of the ketones indicated for fractions 1, 2, 4 and 6 in table 1, mixed melting points were performed with the semicarbazones prepared from the respective fractions and the corresponding known derivatives. None of these mixtures showed any depression in melting point. In order to test the validity of this procedure as a confirmatory test, equal quantities of octanone-2 (melting point, 122° C.) and heptanone-2 (melting point, 123° C.) semicarbazones were intimately mixed and the melting point determined. This mixture gave a melting point of 107° C. or a depression of approximately 15° C. Thus, it would appear that the mixed-melting point procedure was a suitable confirmatory test.

*Control experiment.* It seemed advisable to determine whether compounds of the type isolated in this investigation were present in blue cheese which had not been subject to steam distillation. Blue cheese (0.5 lb.) was macerated with a small volume of ethyl ether and the mixture allowed to stand for several hours,

after which time the ether extract was decanted. This extract was concentrated by evaporating the solvent on a warm water bath. The residue had a strong aroma of blue cheese and gave positive reactions with nitroprusside, iodoform and 2,4-dinitrophenylhydrazine reagents. The results of these tests would indicate that methyl ketones are present in blue cheese prior to steam distillation of the cheese.

#### DISCUSSION

The data of table 1 for fractions 2, 4 and 6 adequately demonstrate the identity of pentanone-2, heptanone-2 and nonanone-2 in the steam distillable material from blue cheese. The data concerning boiling ranges, refractive indices and derivatives are in good agreement with those reported in the literature (3) for the indicated ketones. Fraction 1 contained in addition to pentanone-2, ethyl alcohol which was carried over in the ether used for extraction. Results from the control experiment denote that the methyl ketones identified are present in the cheese prior to steam distillation. The high concentration of methyl ketones observed in the first portion of steam distillate from blue cheese also suggests that heat decomposition of the cheese during distillation is a minor consideration. However, it is conceivable that beta-keto acids, possible intermediates in the formation of methyl ketones from fatty acids, are converted to methyl ketones during steam distillation of the cheese. The extent to which these acids contribute to the total acetone bodies of blue cheese will bear further investigation. Beta-oxidation of fatty acids by molds has been studied and elucidated by Stokoe (6) among others, and interpreted in terms of blue cheese flavor, as related to methyl ketones, by Hammer and Bryant (2). The stages in the beta-oxidation of fatty acids to methyl ketones which have been proposed are first to the beta-hydroxy acid and then to the beta-keto acid which is decarboxylated to yield a methyl ketone and carbon dioxide. The isolation in these experiments of methyl ketones with only odd numbers of carbons is in keeping with characteristics of the beta-oxidation mechanism. Thus, the precursors of acetone, pentanone-2, heptanone-2 and nonanone-2 may be butyric, caproic, caprylic and capric acids of butterfat, respectively.

The fact that no appreciable quantity of any ketone boiling above 200° C. could be recovered in this study would seem to warrant some discussion. Although fraction 7 (boiling range, 200–255° C.) contained small quantities of ketone, it was composed mainly of other materials. These materials might well have been fatty acids, since no measures were taken to remove such compounds. Presence of the high boiling material appears to have been advantageous, since it served as a "pusher" for the methyl ketones during fractional distillation. A further consideration is that only 2 l. of steam distillate were taken from 16 lb., 3 oz. of blue cheese. It is possible that steam distillation to this limited extent was not sufficient to permit recovery of the higher boiling ketones. It also is quite possible that little or no additional ketones were present in the cheese. According to Stokoe (6), no acids above lauric in molecular weight are absorbed by molds; consequently, no ketones above undecanone-2 (boiling point, 223 to 226° C.) are formed. Since these investigations of blue cheese are being

continued, the above matters will receive adequate study in the future. For the present, recovery of the ketones without prolonged and rigorous steam distillation seemed justified.

#### SUMMARY

By means of a steam distillation and ether extraction procedure, it has been possible to recover material from blue cheese containing a high concentration of methyl ketones. By fractional distillation of this material relatively pure fractions of pentanone-2, heptanone-2 and nonanone-2 were obtained. Acetone was identified as a constituent of the ether-extracted steam distillate from blue cheese. These methyl ketones would appear to be formed from the fatty acids in the cheese by beta-oxidation.

Similarity in odor between these ketones, particularly heptanone-2 and that of blue cheese was noted by a number of observers during the course of this investigation. It seems probable that minute quantities of these methyl ketones are the constituents which make the flavor of mold-ripened cheeses distinctly different from the flavor of other types of cheese.

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## STANDARDIZING THE BABCOCK TEST FOR MILK BY INCREASING THE VOLUME OF THE SAMPLE AND ELIMINATING THE MENISCUS ON THE FAT COLUMN

E. O. HERREID,<sup>1</sup> L. H. BURGWARD, B. L. HERRINGTON AND E. L. JACK  
*Illinois, Ohio, New York and California Agricultural Experiment Stations, respectively*

The Babcock test has been used in the dairy industry for nearly 60 yr. During this period of time, there have been no significant refinements in conducting the test, except for more accurate glassware and improved heated centrifuges.

When Babcock (2) contributed his test to the dairy industry, it was sufficiently accurate for most practical purposes; in fact, he did not claim a high degree of accuracy for his test. As the industry became specialized, emphasis was placed on improving the efficiency of dairy plants by more carefully accounting for milk fat in milk and cream purchased and in processed and manufactured products. It is the opinion of students of testing problems that the Babcock method can be made more accurate and thereby be more useful in accounting for milk fat in dairy plants. For this reason a subcommittee was appointed in the American Dairy Science Association to study methods for refining the method, without sacrificing its simplicity and usefulness.

A number of investigators (7) have determined the accuracy of the Babcock method by comparing it with the ether extraction method which is the standard for comparison. The earlier comparisons agreed closely, but those made in recent years show that the Babcock method yields results from 0.05 to 0.07 per cent higher than those obtained with the Roesse-Gottlieb method or its mechanized modification, the Mojonnier method.

Investigators in experiment stations and technicians in commercial laboratories have recognized that the upper meniscus on the fat column is a variable factor in reading the Babcock test for fat in milk. Babcock (2) stated that the lowest and the highest limits of the fat column should be included in the reading and this meant that (3) the reading should be taken at a point where the upper surface of the fat meets the side of the graduated neck and not from the dark line caused by the refraction of the curved surface. For the past 30 yr. this method of reading the Babcock test has been stated by the Association of Official Agricultural Chemists in its Official and Tentative Methods of Analysis (1). Dahlberg (5) and Herreid *et al.* (10) have emphasized the difficulty of determining the exact point at which the fat meets the wall of the graduated neck. Babcock included the meniscus in the fat reading in order to obtain closer

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<sup>1</sup> Chairman and members, respectively, of Subcommittee in the Manufacturing Section of the American Dairy Science Association, to Standardize the Babcock Test for milk fat to agree with the Mojonnier method.

agreement with the ether extraction method and it has been shown (4, 6, 8) that some fat remains in the bulb of the test bottle and is not collected in the graduated neck during the centrifuging process. Including the meniscus compensates for this residual fat.

Evidently the Babcock test yields results that are higher than those obtained with the ether extraction method and the meniscus on the fat column makes it difficult for different technicians to obtain consistent results with the present technique of reading fat tests of milk.

#### PROCEDURE

The Committee decided that the simplest approach to standardizing the Babcock test in order to obtain agreement with the ether extraction method was to increase the volume of the sample of milk and eliminate the meniscus on the fat column. Letters were sent to a number of prominent leaders in the dairy industry to obtain their opinions concerning this method. There was practically unanimous agreement that this is a sound and practical procedure.

TABLE 1

*Reading Babcock fat tests of unpreserved milk with and without the meniscus and comparing them with the Mojonnier method*

Station	No. of samples	Method			Mojonnier	Difference*
		Babcock				
		With	Less	Meniscus		
		meniscus	meniscus			
		(%)	(%)	(%)	(%)	(%)
Ohio	81	4.35	4.21	0.14	4.30	- 0.09
Illinois	44	4.16	4.01	0.15	4.09	- 0.08
Vermont	88	4.26	4.12	0.14	4.20	- 0.08

\* Between Mojonnier and Babcock less meniscus.

The first step was to determine how much the elimination of the meniscus would reduce the Babcock test for milk as compared to the Mojonnier method. This was accomplished by determining the fat content of a number of samples of unpreserved milk with the Babcock method and reading the tests with and without the meniscus. After the fat tests were read by the regular Babcock method, the bottles again were placed in the water bath for 5 min. and they were read immediately after the meniscus had been eliminated with glymol. When the meniscus is eliminated, the results from three experiment stations in table 1 indicate that the Babcock test for milk is reduced on the average by slightly more than 0.08 per cent below the Mojonnier method. Therefore, to get both methods to agree, it will be necessary to increase the weight of the milk samples for the Babcock method by an amount equivalent to 0.08 per cent. Assuming that the milk contains 4 per cent of milk fat, the sample should be increased by 0.36 g.,  $\left(\frac{0.08}{4} \times 18 = 0.36\right)$  making a total weight of 18.36 g. of milk which must be delivered by the pipette.

To determine the amount of milk that might adhere to the pipette, samples were heated to 35 to 36° C. and pipetted into milk test bottles with 18.05 ml. pipettes which had been weighed. The pipettes were allowed to drain for 10 to 15 sec. after free outflow had ceased and the milk in the tip was blown out. They were again weighed to obtain the net weight of milk in each one. The average weight of milk retained by 36 pipettes was 0.13 g., varying from 0.10 to 0.15 g. Consequently, the total weight of milk required for the modified Babcock test is 18.49 g. ( $18.36 + 0.13 = 18.49$ ) for milk containing 4 per cent of milk fat.

Since the capacity of the pipette that will contain 18.49 g. of milk must be known, specific gravity determinations were made on 62 samples of unpreserved milk. The average specific gravity at 35 to 36° C. was 1.025. Consequently, the capacity of the pipette should be 18.05 ml. ( $18.49 \div 1.025 = 18.04$ ). A temperature of 35 to 36° C. was used because preserved composite samples are prepared and pipetted at 35 to 38° C. Therefore, unpreserved milk also should be sampled in this same temperature range.

Increasing the sample by a constant weight of 0.36 g. is not proportional to the fat content of all milk because it is based on the approximate fat percentages of the experimental samples. It is evident that there is a slight bias in favor of the lower testing milks and a slight bias against the higher testing ones, (table 2)

TABLE 2

*The calculated effect on tests of milk of different fat percentages by increasing the weight of the sample 0.36 g.*

Milk fat	Calculated size of sample	Volume of sample at 35-36° C. sp. gr. 1.025*	Calculated error
(%)	(g.)	(ml.)	(%)
5.00	18.29	17.97	- 0.014
4.00	18.36	18.04	0.000
3.85	18.37	18.05	0.000
3.45	18.42	18.10	+ 0.017
3.25	18.44	18.12	+ 0.025

\* Includes 0.13 g. of milk which, on the average, adhered to pipette.

as indicated by theoretical calculations. The error, however, is small and would probably not be measurable in the great majority of cases with the present milk test bottle.

On the basis of the aforementioned preliminary work, the following procedure for the modified Babcock method was adopted for experimental work by the committee: (a) heat preserved and unpreserved milk samples at 35 to 38° C.; (b) mix thoroughly by pouring milk from one container to another three to four times; (c) fill 18.05 ml. pipette so that the upper surface of the milk is at the graduated mark on the draw tube; (d) drain pipette into test bottle for 10 to 15 sec. after free outflow has ceased and blow out last milk in the tip. Cool samples to 20° C. before adding acid; (e) have  $H_2SO_4$  (sp. gr., 1.82-1.83 at 20° C.) at 20 to 22° C. The amount required will vary from 15 to 17 ml., depending on its strength; (f) proceed as in the original Babcock method; (g) add 2 drops



of a colored mineral oil (glymol, sp. gr. not to exceed 0.85 at 20° C.) as each bottle is taken from the water bath at 57.3 to 60° C. The oil must flow down the sides of the neck and not be dropped onto the fat. Measure the fat from the bottom of the column to the fat-glymol line.

The Mojonnier method was used as the standard for comparison and variations for the modified Babcock method indicated as plus or minus. It was conducted as follows: (a) heat milk samples to 35 to 38° C.; (b) mix thoroughly by pouring from one container to another three to four times; (c) weigh the charge of milk directly into tared extraction flask (9); (d) proceed as in the regular Mojonnier method (11).

The different collaborators calibrated their own pipettes, except in some cases the pipettes were calibrated in an experiment station laboratory. The collaborators obtained their own samples for analysis. Replicate samples of milk were not sent to the different collaborators because it is practically impossible to ship samples of preserved milk without destabilizing the fat emulsion. The collaborators represented experiment station and dairy plant control laboratories and state and federal regulatory laboratories. Some collaborators were suggested by the Association of Official Agricultural Chemists.

#### EXPERIMENTAL

The results of comparisons of the modified Babcock method with the Mojonnier method by 14 collaborators on 232 samples of unpreserved milk are shown in table 3. In addition, five collaborators also submitted results for the regular Babcock method. The results submitted by collaborators A to E inclusive, on a total of 135 samples of milk show close agreement for the modified Babcock and Mojonnier methods. With the exception of collaborator J, who made 27 comparisons, the data from the others are insufficient from which to draw conclusions as to their ability to obtain accurate results with the modified Babcock method. Four collaborators mentioned their inexperience with the Babcock method as probably being responsible for the wide variations which they obtained between both methods.

The results of comparisons by five collaborators of the modified Babcock with the Mojonnier method on 66 samples of preserved milk also are shown in table 3. Collaborators B and D obtained close agreement between both methods on 26 samples of milk, while collaborators C, I and K reported mean variations of - 0.03, + 0.04 and - 0.10 per cent, respectively. The standard deviations of the differences between the modified Babcock and Mojonnier methods did not exceed 0.05 per cent for collaborators A to E, inclusive, and they did not exceed 0.1 per cent for any of the other collaborators.

The data submitted by some of the collaborators emphasizes the necessity of standardizing the Babcock method so that it will yield satisfactory agreement with the Mojonnier method. For example, for the regular Babcock and Mojonnier methods on nonpreserved milk, collaborator A obtained a mean difference of + 0.08 per cent, collaborator F a mean difference of + 0.06 per cent and collaborator I a mean difference of - 0.02 per cent.

TABLE 3

*A comparison of the results obtained with the Babcock, the modified Babcock and the Mojonnier methods on samples of nonpreserved and preserved milk by different collaborators*

Collaborator	No. of trials	Regular Babcock	Modified Babcock	Mojonnier	Difference <sup>a</sup> mean	S.D.
		(%)	(%)	(%)	(%)	(%)
<i>Nonpreserved</i>						
A	37	3.58	3.49	3.50	-0.01	0.05
B	24		4.22	4.21	+0.01	0.02
C	24		3.58	3.59	-0.01	0.03
D	20		4.08	4.07	+0.01	0.02
E	30		4.13	4.12	+0.01	0.01
F	12	4.20	4.17	4.14	+0.03	0.02
G	6		4.24	4.22	+0.02	0.04
H	8		3.78	3.76	+0.02	0.01
I	11	3.83	3.81	3.85	-0.04	0.06
J	27		4.70	4.74	-0.04	0.06
K	12		3.81	3.89	-0.08	0.04
L	6	4.00	3.90	4.01	-0.11	0.02
M	9		3.73	3.71	+0.02	0.03
N	6	3.97	3.94	3.95	-0.01	0.05
<i>Preserved</i>						
B	16		4.20	4.20	0.00	0.03
C	24		4.82	4.85	-0.03	0.05
D	10	4.00	3.97	3.98	+0.01	0.05
I	5	3.96	3.94	3.90	+0.04	0.01
K	11		3.71	3.81	-0.10	0.07

<sup>a</sup> Between modified Babcock and Mojonnier methods.

#### DISCUSSION

The results submitted by collaborators A to E, inclusive, on a total of 135 samples of unpreserved milk indicate that the modified Babcock method is accurate. These collaborators were all experienced laboratory technicians. The data from the other nine collaborators, with the exception of collaborator J, are insufficient to prove their ability to obtain accurate results with the modified method.

In the present Babcock test the inclusion of the meniscus is somewhat empirical, that is to include partially void space as part of the fat column in order to compensate for fat, some of which adheres to the walls and shoulder of the test bottle and some of which is probably suspended and dissolved in the acid solution. In the modified Babcock test this empirical value is substituted, in part, with another in that the volume of the sample is increased to compensate for the amount that removal of the meniscus lowers the Babcock test on the average below the Mojonnier method. The chief disadvantage of the modified Babcock test is that it is more difficult to explain the reason for the specified capacity of 0.18 g. (2 ml.) of fat for each per cent on the graduated neck of the test bottle because the weight of the sample of milk is increased from 18 to 18.36 g.

For the most part official methods have lagged in improving testing techniques. This is not intended as a criticism of those responsible for the official methods; in fact, the dairy industry has not requested from the Association of Official Agricultural Chemists more refined techniques for testing milk and milk

products. For example, Official and Tentative Methods of Analysis (1) specifies a sampling temperature of 15 to 20° C. for milk and if lumps of cream are not dispersed, the sample should be warmed to about 38° C., mixed thoroughly and finally cooled to 20° C. before pipetting. The way composite samples are kept in most plants at the present time it is necessary to heat them to about 38° C. to obtain a homogeneous mixture. Cooling the samples back to 20° C. is very apt to result in partial churning of the fat emulsion when they are mixed immediately before being pipetted into test bottles. Consequently, the fat tests of partially churned samples are lowered because clusters of fat adhere to the inner surface of the pipette.

Since it is necessary to heat preserved composite samples at 35 to 38° C. in order to prepare and pipette them accurately, obviously the same technique should be used for unpreserved milk samples. It is not possible to account for fat in dairy plants as accurately when milk purchased is sampled and pipetted at one temperature and when it is sold these techniques are performed at a different temperature. Nevertheless, this is being done where milk is purchased on the basis of the fat content of preserved composites and is sold on the basis of the fat content of unpreserved samples.

In comparing the accuracy of the modified Babcock with the Mojonnier method, it should be recognized that the fatty materials are chemically different and the procedures for estimating them are radically different for each method. In the Babcock method milk fat is released from its emulsion in normal milk by the drastic action of a strong acid and is finally removed from the acid solution by adding water and centrifuging. During its contact with the acid solution, the fat undergoes some chemical changes whereby free fatty acids are formed and possibly some of the fat is sulfonated and water is adsorbed. On the other hand, the solvents used in the Mojonnier method for normal milk extract milk fat without changing it chemically. However, in addition to milk fat a portion of the phospholipids, chiefly lecithin also is extracted. Consequently, fat estimations made by the Mojonnier method are slightly higher than they should be, but the errors are small for milk because the phospholipids are reported to average about 0.04 per cent and not all of them are extracted by the Mojonnier method.

The suggested revisions for the modified Babcock method are simple and fundamentally sound. Only the pipettes will have to be changed and regulatory officials in each state could have them recalibrated. The use of glymol is the only added technique and it has been an accepted part of the Babcock test for cream for about 35 yr.

#### SUMMARY

Standardizing the Babcock test for milk by increasing the volume of the sample and eliminating the meniscus on the fat column is simple, fundamentally sound and accurate as indicated by comparisons with the Mojonnier method on 135 samples of unpreserved milk by five experienced laboratory technicians. It is the judgment of the Committee that the modified Babcock method is better than the method now in use.

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# A STUDY OF THE CAVITATION EFFECT IN THE HOMOGENIZATION OF DAIRY PRODUCTS<sup>1, 2</sup>

C. C. LOO, W. L. SLATTER<sup>3</sup> AND R. W. POWELL<sup>4</sup>

*Departments of Dairy Technology and Mechanics  
Ohio State University, Columbus*

The homogenizer has been in use for more than 50 yr. Various theories have been advanced to explain the phenomenon of homogenization. Impingement has been discussed as a factor in homogenization by Doan and Minster (4), Sommer (19), Doan (5), Farrall (6) and Tracy (21). Explosion of fat globules due to sudden change in pressure was discussed by Farrall (6) and Tracy (21). Shearing of fat globules by layers of liquid travelling at different velocities at the valve clearance was discussed as a responsible factor of homogenization by Sommer (19), Farrall (6) and Tracy (21). The doubtful nature of these theories was discussed by Sommer (20) in 1946.

Conventional homogenizers are so constructed that the product attains a very high velocity on flowing through the homogenizer valve. From hydraulic principles it can be expected that there will be a corresponding drop in pressure at the sharp entrance to the valve clearance. Should the pressure drop to the value of the vapor pressure, vapor cavities will form in the flow giving rise to the phenomenon known as "cavitation." The destructive effect of cavitation (10, 11, 12, 13, 14, 15) suggests that it might be a very important factor in homogenization. In fact, cavitation has been demonstrated to be responsible for the production of an emulsion of two immiscible liquids by intense sonic vibration by various authors, notably Gaines (8), Chambers (1, 2) and Sollner (16, 17). Therefore, it seemed that the cavitation effect might be a possible factor in the homogenization of dairy products. A study of its role in homogenization should aid in explaining the mechanism of this process.

## EXPERIMENTAL PROCEDURE

A hydraulical analysis of the flow through a Cherry-Burrell 125 viscolizer valve was conducted to determine whether cavitation would occur. Mathematical expressions of the relationships between the valve clearance were derived. Such theoretical relationships were checked by actual measurement of the corresponding valve clearance and applied pressure from which the lowest pressure at the valve clearance was calculated. The original valve was modified to show the effect of cavitation in the homogenization process. The original and the modified valves are shown diagrammatically in fig. 1.

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<sup>2</sup> Approved for publication as Scientific paper 1-50. Department of Dairy Technology, Ohio State University.

<sup>3, 4</sup> Departments of Dairy Technology and Mechanics, respectively.

The following modifications were made to reduce the applicability of the prevailing theories of homogenization: (a) Most of the sections which tend to create shearing were cut away; (b) the wall surrounding the valve was made at a greater distance from the valve clearance and inclined at an angle to the stream to reduce the intensity of collisions of the fat globules on the wall.

The relationship of the valve clearance and applied pressure also was determined experimentally for the modified valve. Calculations based on these experimental values were made to ascertain whether cavitation would occur in this valve.

The original valve and the modified valve were used to homogenize four lots of milk and one lot of cream of varying fat content. Milk was pasteurized at 143° F. for 30 min. and then homogenized at  $140 \pm 2^\circ$  F. The cream was pasteurized at 158° F. for 30 min. and homogenized at that temperature. The milk or cream was homogenized at pressure intervals of about 300 lb. from

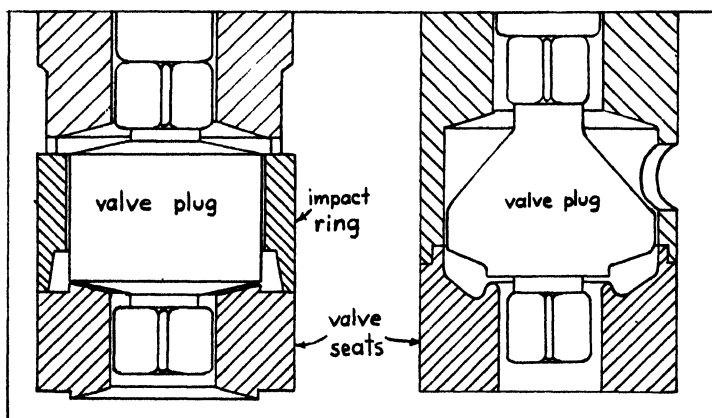


FIG. 1. Cross-sectional views of the Cherry-Burrell 125 viscolizer valve assembly (left) and the modified valve assembly (right) used in the investigation.

0 to 2500 lb. per in.<sup>2</sup> Three methods were used to measure the degree of homogenization of milk, namely, the microscopic measurement of fat globule size, the determination of the United States Public Health Service (U.S.P.H.S.) index (22) and a specially developed centrifugal pipette method. The latter method determines the equivalent specific surface area of the fat phase. Only the microscopic and the centrifugal pipette methods were used on cream.

In the microscopic method, glycerine was used as part of the diluent as was used by Farrall *et al.* (7). The fat globules were stained with Nile blue sulphate, as suggested by Cole and Smith (3). The microscope used was equipped with a 15x eyepiece, a 90x objective and a micrometer of 200 divisions to 1 cm. The micrometer was calibrated to read  $0.65 \mu$  per division. Fat globules were classified according to size. The first class included fat globules less than  $1.5 \mu$  and other classes were established at  $1 \mu$  levels, *i.e.*, 1.5–2.5, 2.5–3.5, etc. About 160 to 170 globules from widely scattered fields were considered

sufficient to yield representative results. Since stability of a dispersion is mainly a surface effect, the roots of the mean square of the diameters of the globules were calculated to indicate degree of homogenization.

In determining the U.S.P.H.S. index of the degree of homogenization, the storage temperature used was 40° F. A siphon which had one leg long enough to reach the bottom of the quart bottle was used to determine the fat content. Mixing of milk and acid for 3 to 5 min. with a mechanical shaker assured clear fat columns.

The centrifugal pipette method was developed to give numerical indication of the degree of homogenization over a wide range. The principles underlying this method were to increase the rate of globule migration by means of centrifugal force and to obtain a more nearly representative sample of the top layer by setting a special pipette in the sample at a certain depth during the entire centrifuging period. Knowing the average fat test of the sample and that of the top layer after centrifuging, the rate of fat globule migration could be calculated. Neglecting the effects of emulsification and electrical charges, the equivalent specific surface area of the fat phase (square centimeters per gram fat) could be determined.

The apparatus used in the centrifugal pipette method include 20 × 150 mm.-test tubes with a line etched at 1.6 cm. from the top, special pipettes, numbered and tared (see fig. 2a), special pipette holders for weighing the pipettes (see fig. 2b), Babcock centrifuge (deep pocket type), and Mojonnier testing equipment.

The procedure of the centrifugal pipette method is as follows: (a) Fill the test tube with well mixed milk sample to about the level mark at 1.6 cm. from the top. (b) Adjust the temperature to 70° F. by immersion in water bath for about 5 to 7 min. (c) Place the pipette into the test tube and adjust the level of milk to the level mark. (d) Centrifuge in a Babcock centrifuge for exactly 8.5 min. Previous to use, the interior temperature of the centrifuge also should be adjusted to about 70° F. (e) Remove the pipette with its contents as follows: First, stop the short end of the pipette with the thumb of one hand while the other hand holds the test tube. Invert the whole system gently, pouring the milk from the test tube while keeping the pipette stopped at the short end. Then withdraw the pipette downward, using care so as not to touch it on the wall of the test tube which may carry a heavy cream layer. (f) Keep the pipette in a tilted position, allowing the milk inside the pipette to subside slightly toward the bend but not so much as to reach the thumb stopping the hole. Wipe the outside clean with cheese cloth. If the outside of the pipette is coated heavily with cream, it may be washed carefully under a warm water tap and then wiped clean and dry. (g) Use the special pipette holder to weigh the pipette and milk on an analytical balance. Drain the milk into a Mojonnier extraction flask. Rinse out the pipette three to four times with about 0.5 ml. distilled water at a time. A medicine dropper is helpful for rinsing. (h) Add water to the extraction flask to make up the volume to 10 ml. and then proceed according to the method of testing milk

on a Mojonnier tester. (i) In expressing the results, let the per cent fat migrated out of the lower 8 cm.-portion in the test tube during the 8.5 min. be designated as the centrifugal pipette method index  $I_{cp}$ ; the test of the milk in the pipette,  $F_t$ ; and the average test of the original milk,  $F$ . Then

$$I_{cp} = \frac{5.4 (F_t - F)}{8 F} \times 100$$

By applying Stokes' law, the equivalent diameter of the fat globules can be calculated and from this value the equivalent specific surface area may be obtained.

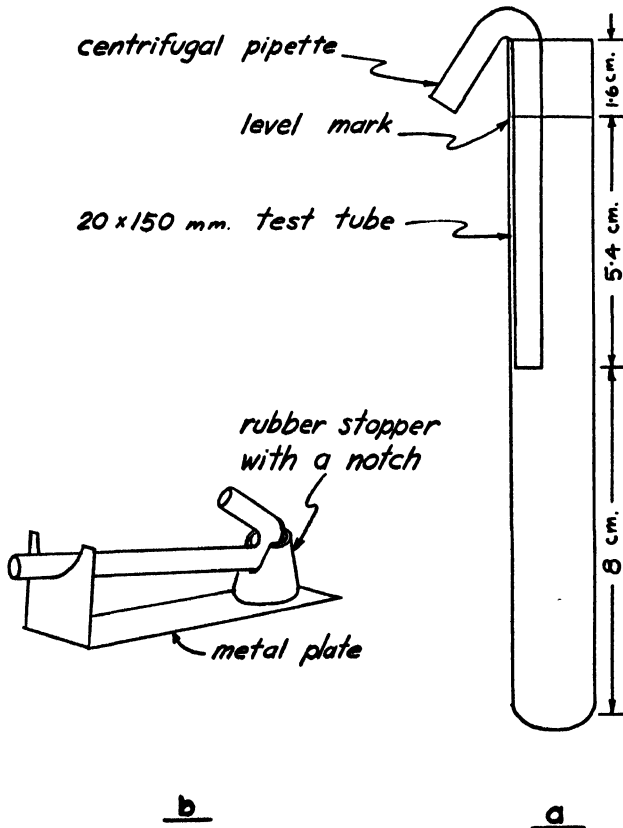


FIG. 2. The centrifugal pipette and the special test tube (a) and the pipette holder (b) used in the centrifugal pipette method of determining the degree of homogenization.

The equivalent specific surface area =  $1.11 \times 10^5 (I_{cp})^{-1}$  cm.<sup>2</sup> per g. For products of which the continuous phase has specific gravities and viscosities different from skimmilk, the above formula for the equivalent specific surface area should be modified accordingly. The formula was plotted on charts for quick conversion operation.



The U.S.P.H.S. index  $I_{PH}$ , the equivalent specific surface area of the fat phase and the root-mean-square diameter of the fat globules were plotted against applied pressure on separate graphs. From the graphs, the pressures at which an  $I_{PH} = 10$ , or an equivalent specific surface area of  $4 \times 10^4$  cm.<sup>2</sup> per g. (this arbitrary value of equivalent specific surface area was found to correspond satisfactorily to well homogenized milk) could be determined for both values. The reciprocals of these pressures were used to represent the mechanical efficiencies of the valves for the purpose of comparing their performances.

#### RESULTS

*The determination of the occurrence of cavitation phenomenon in homogenization.* A diagrammatical view of the Cherry-Burrell 125 viscolizer valve

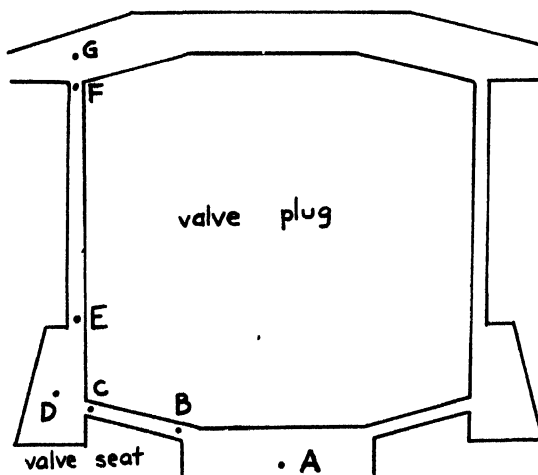


FIG. 3. Simplified diagram of the Cherry-Burrell 125 viscolizer valve.

assembly is shown in fig. 3. The flow between  $B$  and  $C$  was found to be turbulent while that between  $E$  and  $F$  was found to be in the critical range between turbulent and laminar when ice cream mix was used. The following expressions were derived to represent the relationship between the applied pressure and the valve clearance:

When the flow was assumed laminar between  $E$  and  $F$ ,

$$P_A = 417r^3 + 328r^2 + 212.9 \text{ lb./in.}^2 \quad \text{Eq. 1}$$

$P_A$  = applied pressure;  $r$  = one half of the valve clearance in thousandths of an inch. When the flow was assumed turbulent between  $E$  and  $F$ ,

$$P_A = 417r^3 + 328r^2 + 272.0 \text{ lb./in.}^2 \quad \text{Eq. 2}$$

The lowest pressure that would occur in the flow was found to be at the vena contracta (the contraction of the flow at a sudden constriction in the passage) at the entrance of the valve clearance. It could be calculated from the following equation:

$$P_{vc} = P_A - 1198.3r^2 \text{ lb./in.}^2 \quad \text{Eq. 3}$$

From equations 1, 2 and 3, it could be seen that a turbulent flow from *E* to *F* would give the pressure at the vena contracta at the valve clearance a higher value than would be obtained by laminar flow. Theoretically, if the higher value would decrease to the vapor pressure of the ice cream mix when the applied pressure was at a value less than normal homogenization pressure, cavitation should occur during homogenization. Therefore, equation 1 was eliminated and only equations 2 and 3 were used to represent the relationship between valve clearance, applied pressure and the pressure at the vena contracta at the valve clearance. Curves 2 and 3 in fig. 4 are equations 2 and 3 plotted with pressure against valve clearance. These curves indicate that the pressure at the vena contracta dropped to the vapor pressure of the ice cream mix when the homogenizing pressure was at about 540 lb. per in.<sup>2</sup> Theoretically, then, these calculations suggest that cavitation should occur in the original valve.

The equations for skimmilk and water also were derived and are presented below:

For skimmilk:

$$P_A = 4 - 2r^{-3} + 316r^{-2} + 153 \text{ lb./in.}^2 \quad \text{Eq. 4}$$

$$P_{vc} = P_A - 1150r^{-2} \text{ lb./in.}^2 \quad \text{Eq. 5}$$

For water:

$$P_A = 387r^{-3} + 304r^{-2} + 142 \text{ lb./in.}^2 \quad \text{Eq. 6}$$

$$P_{vc} = P_A - 1110r^{-2} \text{ lb./in.}^2 \quad \text{Eq. 7}$$

Water and skimmilk were used to determine experimentally the relationship between valve clearance and applied pressure for the original valve. The results are plotted in fig. 4 together with the theoretical valve clearance-applied pressure curves. The experimental curves follow the theoretical curves closely except at the lower pressures. By putting a series of experimental values of the applied pressure and the valve clearance into equations 5 and 7, it was found that the pressure at the vena contracta at the valve clearance tended to decrease to zero at an applied pressure of about 900 lb. per in.<sup>2</sup> when skimmilk was used and at an applied pressure of about 700 lb. per in.<sup>2</sup> when water was used. These experimental results also suggest that cavitation should occur in the original valve.

The relationship between the valve clearance and applied pressure for the modified valve was determined using milk. The results are plotted in fig. 4 as curve 8. Applying Bernoulli's equation to the flow at the valve clearance, it was found that the pressure at the valve clearance tended to drop to zero when the applied pressure reached 200 lb. per in.<sup>2</sup> Therefore, cavitation also should occur in the modified valve at higher pressures.

*Comparison of the performances of the modified and the original valves.* The typical curves showing the performances of the modified and the original valves as determined by the centrifugal pipette method, the U.S.P.H.S. index method and the microscopic method are shown in fig. 5. Those curves obtained by means of the centrifugal pipette method and the U.S.P.H.S. index method showed distinct differences between the performances of the two valves and permitted easy determination of the ratio between their mechanical efficiencies. Those curves

obtained by means of the microscopic measurement of globule size revealed only slightly greater reduction in the globule size by the modified valve. The curves became level rapidly to the right, hence it was difficult to determine the ratio between the mechanical efficiencies of the two valves by the microscopic method.

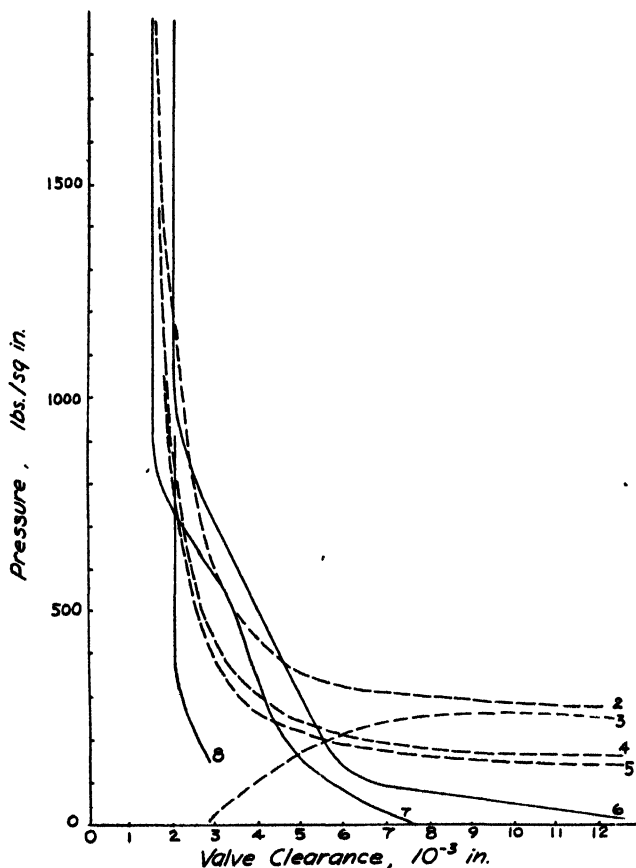


FIG. 4. Curves showing the relationship between applied pressure, the pressure at the vena contracta at the valve clearance and the valve clearance. Curve 2—the theoretical curve of applied pressure *vs.* valve clearance of the original valve using ice cream mix. Curve 3—the theoretical curve of the pressure at the vena contracta at the valve clearance *vs.* valve clearance of the original valve using ice cream mix. Curves 4 and 5—the theoretical curves of the applied pressure *vs.* valve clearance of the original valve using skimmilk and water, respectively. Curves 6 and 7—the experimental curves of the applied pressure *vs.* valve clearance of the original valve using skimmilk and water, respectively. Curve 8—the experimental curve of the applied pressure *vs.* valve clearance of the modified valve using milk.

Table 1 presents the ratio of the mechanical efficiencies of the modified and the original valves from four trials of milk and one trial of cream, as determined by the centrifugal pipette method and the U.S.P.H.S. index method. The centrifugal

pipette method revealed 27 to 85 per cent higher mechanical efficiency in the modified valve, while the U.S.P.H.S. index method revealed 6 to 47 per cent higher efficiencies in the modified valve. Hence, the modified valve was found to produce the same degree of homogenization at lower pressures than required with the original valve.

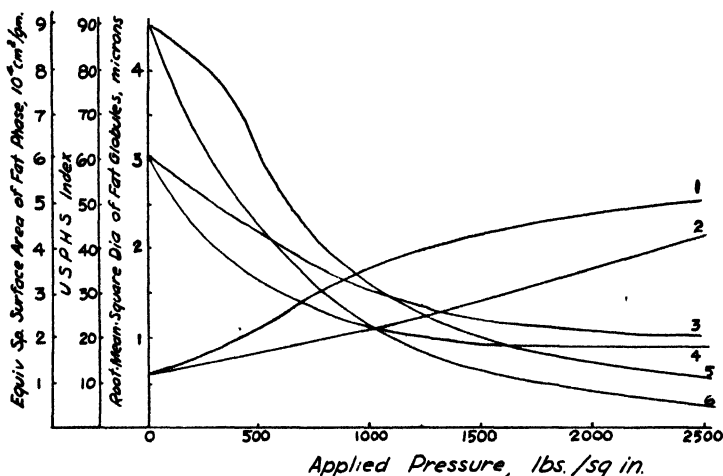


FIG. 5. Typical curves showing the performances of the original and the modified valves. Curves 1 and 2—curves of the modified and the original valves, respectively, as determined by the centrifugal pipette method (equivalent specific surface area of the fat phase *vs.* applied pressure). Curves 3 and 4—curves of the original and the modified valves, respectively, as determined by the microscopic method (root-mean-square diameter of the fat globules *vs.* applied pressure). Curves 5 and 6—curves of the original and the modified valves, respectively, as determined by the U.S.P.H.S. index (U.S.P.H.S. index *vs.* applied pressure).

#### DISCUSSION

The experimentally determined relationship of the valve clearance and the applied pressure agreed favorably with the theoretical values, as may be seen in fig. 4. The relatively large disagreement between the theoretical and the experimental curves at lower pressures probably was due to the fact that the pressure gauge was primarily made to read accurately at normal homogenization pressures and not for pressures as low as 500 lb. per in.<sup>2</sup> or less.

The valve clearance measurements showed that at normal homogenization pressures the valve clearance of both valves would be approximately 0.002 in., which was about ten times as large as the average diameter of fat globules in unhomogenized products.

In discussing the explosion theory, Sommer (20) pointed out that the low compressibility of the liquids rendered the occurrence of explosion doubtful. This reason appears inadequate in itself, for a liquid may change into a vapor state which is capable of expanding or contracting rapidly. This is supported by the phenomenon of "rebound" of vapor bubbles observed by Knapp and Hollander

(9). However, an explosion due to change of physical state is not likely to occur in milk fat, because the serum phase which has a much higher vapor pressure will change state first and, thus maintain the pressure at a rather constant level.

The wall surrounding the valve slit of the modified valve was designed to incline at an angle to the emerging stream and at a distance farther away than in the original valve, and yet the modified valve showed a higher mechanical efficiency.

Both theoretical and experimental results obtained using the original valve indicated that cavitation should occur in the flow when the homogenizer was working at normal homogenization pressures. Calculations based on actual meas-

TABLE 1

*Comparisons of the performances of the modified and the original valves as determined by the centrifugal pipette method and the U. S. P. H. S. index*

Trial no.	Product	Fat test of the product	Mech. eff. of the orig. valve Mech. eff. of the mod. valve	
			As determined by	
			Cent. pipette method	U. S. P. H. S. index
		(%)		
1	milk	3.82	100	100
			183	147
2	milk	4.59	100	100
			141	119
3	milk	4.47	100	100
			138	113
4	milk	4.07	100	100
			127	106
5	cream	20.29	100	—
			185	—

urements of valve clearance of the modified valve also indicated the occurrence of cavitation when this valve was used. Sufficiently large Reynold's numbers indicating turbulent flow were obtained for the original valve.

The results obtained from valve performance comparisons indicated that the modified valve had higher mechanical efficiency than the original valve. The higher mechanical efficiency of the modified valve seemed to indicate that cavitation might be one of the important factors of homogenization, for the modified valve was designed in such a way as to reduce the applicability of the prevailing theories of homogenization and to bring about a definite cavitation phenomenon.

When an homogenizer is being operated, a sudden development of a hissing noise occurs when the pressure rises to a certain value. Such a hissing noise has been described by Sollner (18) and Kornfeld and Suvorov (10) as an indication of cavitation phenomenon in a flow.

The role of cavitation in the homogenization process may be suggested as follows: When the product reaches the vena contracta at the valve clearance the

pressure drops to the vapor pressure of the product due to the tremendous gain in velocity. At this point cavitation begins. The numerous cavities, most of which develop at the fat-serum interfaces, are swept into a region where the pressure becomes higher due to either enlargement of the flow passage or pressure waves created by the collapse of other cavities. Here the vapor phase can no longer exist and the cavities collapse instantaneously, resulting in numerous blows that shatter the fat globules. Also, the instantaneous formation of cavities may cause numerous spots of dehydration which in turn cause local temporary increase in acidity and the collapse of the cavities produces high frequency pressure waves, shock and local development of heat. This suggests that cavitation also might be involved in other phenomena related to homogenization, such as viscosity change, curd tension reduction, fat clumping, antioxidation effect, denaturation of the proteins, etc.

#### CONCLUSIONS

The analysis of the flow of liquids through a homogenizer valve assembly indicated that cavitation should occur during normal homogenization operations.

An homogenizer valve modified to produce cavitation and to reduce the applicability of some of the prevailing homogenization theories had a higher mechanical efficiency than the original valve.

Cavitation has been suggested as one of the important factors responsible for the homogenization of dairy products.

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# A MICROMETHOD FOR ROUTINE DETERMINATIONS OF FAT IN SKIMMILK AND NONFAT DRY MILK SOLIDS

BURDET HEINEMANN AND M. ROBERT ROHR

*Producers Creamery Co., Springfield, Mo.*

While modifications of the Babcock test have been used successfully for testing buttermilk and whey, no satisfactory Babcock test has been published for testing skimmilk for fat. The modification using butyl alcohol presents the most consistent results. However, these results, even when obtained from the most careful analysis, still are as little as one-tenth of the amount of fat measured by the Mojonnier method. If, for example, a Babcock result is 0.01 per cent, the Mojonnier method may result in 0.05 to 0.10 per cent fat. Therefore, processing losses may vary as much as 100 per cent without detection by the Babcock test. The Mojonnier method, while measuring all of the fatty extract, takes about 45 min. to run and hence, is not suitable for a routine control test.

In order to have a suitable control test, a nephelometric determination (2) was developed. This method required about 20 min. to complete and the accuracy was satisfactory. However, further studies resulted in a method based on the work of Jones (3). He showed that a method could be employed to measure the quantity of fat in an ether solution by the area of its monolayer formed on a water-sulphuric acid film with a surface tension of 20 dynes per square centimeter. This method was found to yield results in 12 min.—about the same length of time involved in a Babcock test.

## APPARATUS AND MATERIALS

*Ultramicro-pipettes.* Two types of pipettes are satisfactory for use. The Kirk pipette is commercially available.<sup>1</sup> These were graduated to contain 0.0075 ml. If the pipette is graduated "to contain," it must be rinsed at least twice with pure petroleum ether, placing the rinsings in the center of the spread. For routine use, however, pipettes were prepared from Breed and Brew pipettes by drawing the tip to about 1 or 2 mm. Some care must be exercised in the drawing process. The bore should not be small enough to allow the mercury to separate. The pipette may be calibrated with mercury on a "to contain" basis. It also may be calibrated with benzyl benzoate on a "to deliver basis." The work reported below was done with a pipette calibrated to deliver 0.0072 ml. Several pipettes have been made and calibrated with benzyl benzoate ranging from 0.0072 to 0.0081 ml. and all found suitable from the standpoint of accuracy. In use, a rubber or plastic hose about 1 ft. long is attached to the end of the pipette opposite the tip for convenience in drawing in the sample and expelling it. The pipette is rinsed in pure petroleum ether before and after each use.

*Dish.* An 8-in. pyrex pie dish is painted on the inside bottom with black asphalt paint or black enamel. The dish then is coated on the inside with a thin

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<sup>1</sup> Microchemical Specialities Co., Berkeley 3, Calif.



layer of hard paraffin. According to Harkins (1) the paraffin prevents contamination of the acid solution by metallic ions dissolved from the glass. Under routine conditions, and using pyrex glass, this error, if any, is negligible. Continued use of oil gradually dissolves the paraffin on the rim of the dish. For these reasons and for the purpose of this test, sufficient melted paraffin is poured on the inside bottom of the dish to completely cover the paint. The sides and rim are left uncoated. The prepared dish is placed on a leveling table  $9'' \times 9''$  equipped with three leveling screws. The leveling table, in turn, is placed in a tray  $10'' \times 14'' \times 1.5''$  deep.

*Tracing glass.* The edges of a pane of single strength glass about  $14'' \times 20''$  are taped with adhesive tape to allow safe handling. (Transparent plexiglass is not satisfactory.) The tracing glass is laid on the edges of the  $10'' \times 14'' \times 1.5''$  tray and the leveling screws of the leveling table adjusted so that the edges of the pie plate are separated from the tracing glass by about  $0.25''$ . This distance should be as small as possible to prevent parallax, yet not close enough for the surface of the acid solution to accidentally contact the glass while tracing.

*Light source.* A  $12'' \times 12''$  sheet of white opaque plexiglass or heavy white cardboard is positioned almost parallel to and directly above the tray. The height is adjusted to clear the head of the observer. A spotlight (150 watt) is placed to the rear of the tray and directed toward the sheet of white plexiglass. Adjustments are made in the light and reflector until the observer, by leaning over the dish filled with acid solution, may see a uniform white reflection from the white sheet above him.

*Tuberculin syringe.* A 1-ml. tuberculin syringe is fitted with a no. 27 hypodermic needle. The point of the needle is rounded off. The syringe is filled with prepared oil and the tip of the needle held to the surface of the acid solution in the center of the dish. As the piston is very slowly compressed, the oil spreads over the surface with a play of colors. The first color is a brown-gold, followed in turn by purple, blue, green, yellow and red, as first order colors. With the continued addition of oil, the spectrum is completed again as second order colors. Further addition of oil causes the colors to proceed into their third order. When the third order green appears, the addition of oil is stopped and the surface is ready for the deposition of the measured quantity of ether solution of the fatty extract.

*Acid solution.* While Jones (3) specified the use of 0.3 per cent  $\text{H}_2\text{SO}_4$ , a 0.2 per cent solution of glacial acetic acid in distilled water was found preferable. The acid is less corrosive and the colors formed during the deposition of the prepared oil are more easily discerned. Another advantage is that the deposition of oil may be stopped and started without forming clearly defined colors, while this is not the case with  $\text{H}_2\text{SO}_4$ .

*Oil.* Jones specified any good grade of lubricating oil which had been oxidized by heating at  $300^\circ \text{C}$ . for 8 hr. On several oils heated in this manner, tarry residues were obtained which did not spread. The oil used was heated at  $300^\circ \text{F}$ . and air bubbled through during the heating period. At intervals of 0.5 hr., a portion was removed and cooled. This portion was tested for its ability to spread. The oil

used was heated under these conditions for 1.5 hr. However, it was found that each oil must be standardized to yield areas having a factor of 0.9  $\gamma$  of fatty extract per square inch.

*Planimeter.* Two different planimeters were used in this study. It appears that any planimeter reading in square inches with an accuracy of 0.02 in.<sup>2</sup> would be satisfactory.

*Parchment paper.* After the spread is made, the tracing glass is placed over it and the outlines of the spread traced with a china marking pencil having a sharp point. Parchment paper (as used for wrapping butter) or any thin tracing paper is placed over the tracing glass and the area copied to the paper. The paper then is tacked on a drawing board and the area measured with a planimeter.

*Other equipment.* Torsion balance, 10 mg. sensitivity; hot plate; 4 oz. oil sample bottles, tall form; tared Mojonnier fat dishes with lid having a  $\frac{3}{8}$ " hole near one edge; 10"  $\times$  2" double strength glass slide; two 25-ml. Machlett pipettes. The Machlett pipettes must have well fitted stop cocks which require no lubricant. No lubricant has been found which may be safely used. Use of the Silicone "greases" produce an uncontrolled error due to their high solubility in ether.

#### METHOD

Weigh or pipette 4.00 g. of skim milk into a 4-oz. oil bottle or Mojonnier fat extraction tube. For nonfat dry milk solids weight 4.00 g., transfer to a 100-ml. volumetric flask, shake gently to avoid foam and make to the mark with distilled water. Transfer 10 ml. of this solution to the oil bottle or extraction tube. Add 6 ml. of water to the skim milk and add no water to the nonfat dry milk solids. Then, add 1.5 ml. concentrated  $\text{NH}_4\text{OH}$  and shake. Add 10 ml. ethyl alcohol and shake. Using Machlett pipettes, add 25 ml. of ethyl and 25 ml. of petroleum ether, shaking 1 min. after each addition. Allow to stand or centrifuge until the layers have separated. Using a 25-ml. volumetric pipette (which has a rubber stopper on the delivery end to prevent too deep an insertion of the tip into the ether layer), transfer 25 ml. to a Mojonnier fat dish and evaporate to dryness. Cool the pan in water and wipe dry with a clean towel. Cover the pan with an aluminum lid containing a hole large enough to insert a pipette. Place on a Torsion balance and balance. (A butter moisture balance is convenient). Place 2.5 g. on one side and add petroleum ether in slight excess—about 4.1 ml. Rotate the cup, holding it lightly in the fingers so as to avoid transfer of body heat.

At this point, prepare the surface of the acid solution by passing the glass slide across the surface three or four times, wiping the edge of the slide with a clean towel after each passage. The temperature of the solution should be between 20 to 30° C. When the surface shows only a few streaks of gold-brown color, it is sufficiently clean to start the addition of oil. After the third order green color is reached, return to the pan on the Torsion balance, add additional petroleum ether to bring the weight to 2.50 g., quickly rotate, insert pipette and withdraw the volume to be spread. Bring the ether solution exactly to the mark on the pipette. Hold the tip of the pipette at the surface and expel the solution

gently, blowing the last drop under the surface. (If a "to contain" pipette is used, it is necessary to rinse it twice with pure petroleum ether at this point transferring the rinsings to the center of the spread. This results in a more irregular figure, making tracing errors larger. A "to contain" pipette may be calibrated more accurately, however.)

The tracing glass then is placed over the surface and the outlines of the figure traced on the glass. This figure is, in turn, traced on parchment paper and the area measured with a planimeter. For routine work, three tracings are made from the figure on the surface, their areas averaged and the fat computed from the average.

The formula used for computation for skimmilk is:

$$\frac{(\text{aliquot}) (\gamma/\text{in.}^2) (\text{area}) (\text{ml. ether solution})}{(\text{ml. spread}) (\text{wt. sample in grams}) (10^4)} =$$

$$\frac{(2) (0.90) (\text{area}) (3.94)}{(0.0072) (4) (10^4)} = (\text{area}) \cdot (0.0246) = \text{per cent fat}$$

The value of the aliquot is 2 since 25 ml. of the combined petroleum and ethyl ether were removed from a total of 50 added to the sample. The value of  $\gamma/\text{in.}^2$  was determined experimentally as outlined below. According to the literature, this value varies with the nature of the fatty substance spread. The specific gravity of the petroleum ether was determined to be 0.635 at 21° C. Since 2.50 g. were used, this is equivalent to 3.94 ml. The pipette used was found to deliver 0.0072 ml. and the weight of the sample for skimmilk was 4 g. In the case of nonfat dry milk solids, the sample weight is 0.4 g. and the calculation of the per cent fat from the area then becomes:

$$(\text{area}) \cdot (0.246) = \text{per cent fat}$$

## RESULTS

*Determination of  $\gamma/\text{in.}^2$ .* The data are limited geographically and seasonally. A total of 32 samples of fatty extract obtained from the Mojonnier test of fresh skimmilk, buttermilk, whole milk and butter during January, February and March was examined. The fatty material was dissolved in petroleum ether to give solutions containing from 0.4 to 5.5 mg. per ml. A 0.0072 aliquot was spread and the area measured. The factor was determined as follows:

$$\frac{(1000 \times \text{mg./ml.}) (0.0072)}{\text{area in in.}^2} = \gamma/\text{in.}^2$$

The mean was 0.90 and the standard deviation was  $\pm 0.07$ . There was no significant difference among the fats from the dairy products examined. This is somewhat surprising, since buttermilk contains the highest percentage of phospholipids which are surface active.

*Recovery.* The method of extraction is the same as reported previously in connection with a nephelometric method for determining fat in skimmilk (2). However, further checks were made by testing the same sample for fat eight times using the above method. Eight 4-g. aliquots of the same sample also were extracted by the standard Mojonnier method adding 5 g. of ether to the dried fatty extract (to compensate for twice as much fatty residue in the pan) and

spreading a 0.0072-ml. portion. The average area of the eight portions extracted once and a 25-ml. aliquot of the ether extract taken was 3.25 in.<sup>2</sup> with a standard deviation of  $\pm 0.11$ . The average area of the eight portions extracted twice and the ether decanted to the cup was 3.24 in.<sup>2</sup> with a standard deviation of  $\pm 0.08$ .

*Precision of the proposed method.* When the same sample of skimmilk was tested eight times under ideal conditions by one of the authors, the coefficient of variation was found to be 2.95 per cent. The other author obtained a value of 2.67 per cent.

*Comparison between the standard Mojonnier method and proposed method for skimmilk.* Forty-nine random samples of skimmilk were tested individually by both methods. The mean per cent fat as obtained by the Mojonnier test was 0.0837. The mean per cent fat as obtained by the proposed method was 0.0824. The range in fat by the Mojonnier test was 0.060 per cent to 0.165 per cent. The standard error of the difference between tests was  $\pm 0.012$  per cent.

*Comparison between the standard Mojonnier method and proposed method for nonfat dry milk solids.* Eighteen random samples ranging from 0.66 to 1.35 per cent were tested individually by both methods. The Mojonnier test averaged 1.06 per cent while the proposed method averaged 1.050 per cent. The standard error of the difference between methods was  $\pm 0.124$  per cent.

*Comparison between the standard Mojonnier method and proposed method for nonfat dry milk solids under routine conditions.* Various members of the laboratory staff made standard Mojonnier tests on nonfat dry milk solids. The same individual usually did not make both the Mojonnier and the proposed test on the same samples. Samples were not tested in duplicate. Twenty-two samples were compared. The average per cent fat by the Mojonnier was 0.927 and by the proposed method was 0.897 per cent. The standard error of the difference between tests was  $\pm 0.104$ . These samples ranged from 0.78 per cent to 1.13 per cent fat by the Mojonnier method.

#### DISCUSSION

The largest single error in the proposed method (hereafter referred to as the "drop test") occurs when the outline of the spread is traced to the tracing glass. The coefficient of variation calculated from tracing the same spread eight times is about 2.5 per cent. In order to reduce this error, the spread is traced three or more times and the average area taken. The next important source of error is in the preparation of the surface for the spread. When a second order green color was used, the coefficient of variation of eight spreads was found to be 10.9 per cent, while with a third order green color, the coefficient of variation was 2.5 per cent. While a few oils other than oxidized lubricating were tried, none was found superior. Temperature of the acid solution apparently is not a factor as long as it is between 20 and 30° C. The concentration of acid likewise does not appear to be important as long as it is about 0.2 per cent. Acetic and sulphuric acid were the only acids tried and, since results with acetic acid were obtained which were satisfactory for routine work, no other acids

were investigated. The coefficient of variation found by calibrating the same pipette with benzyl benzoate on a "to deliver" basis was 0.8 per cent. Pipettes may be calibrated "to contain" with mercury to a higher degree of accuracy. "To contain" pipettes, however, are not as satisfactory for routine work. Careful work by one author using a "to contain" and a "to deliver" pipette resulted in a coefficient of variation for eight spreads of 1.7 per cent for the former and 1.6 per cent for the latter. Tracing from the glass to paper results in a small error of less than 1.0 per cent. Planimeter measurements made of the same tracing on paper eight times resulted in a coefficient of variation of 0.6 per cent. The error resulting from weighing the ether on a Torsion balance was estimated to be less than 1 per cent. The change in weight as determined on an analytical balance was about 2 mg. per sec. and only a few seconds elapse from the time the pan is balanced until the 0.0072-ml. aliquot is taken. Extraction errors for the "drop test" were not adequately determined, since a microbalance was not available. Averaging of a large number of results does indicate that extraction usually is complete. Oxidation of the fatty extract in the pan while on the hot plate was found to be of a negligible nature. The effect of seasonal and geographical variations in the composition of the fat was not determined.

Under routine conditions, the "drop test" may be completed in 12 to 13 min. Some experienced operators may estimate the fat surprisingly well by visual measurement of the area of the spread. The difference between a fat test on skimmilk of 0.07 and 0.09 per cent is easily recognized by judging the area. If such an approximate "high or low" value is desired, results may be secured in about 10 min.

Under ideal conditions, the coefficient of variation is around 2.5 per cent, under routine conditions this value is almost twice as great. For practical purposes, the 25-ml. aliquot of a single extraction evaporated to dryness, ether added, the aliquot spread once, the area traced to glass three times and the per cent fat calculated from the average area will yield a result within  $\pm 0.01$  per cent fat of the true value for skimmilk and  $\pm 0.10$  per cent fat for nonfat dry milk solids. These values approximate those obtained by the Mojonnier method. Ten aliquots of the same sample of skimmilk tested by the Mojonnier had a mean of 0.055 per cent, a standard deviation of  $\pm 0.0048$  per cent and the coefficient of variation was 8.7 per cent. On ten aliquots of the same skim tested by the "drop test" the coefficient of variation was 7.5 per cent, the mean was 0.052 per cent and the standard deviation was  $\pm 0.0039$  per cent. These results probably represent the maximum errors obtained in both methods, since the quantity of fat in this sample was low. When a sample of nonfat dry milk solids was tested seven times by different individuals using the Mojonnier test, the mean was 0.96 per cent, the standard deviation was 0.092 per cent and the coefficient of variation was 9.6 per cent. The same sample of nonfat dry milk solids was tested by the "drop test" by different individuals. The mean of these results was 0.96 per cent, the standard deviation was 0.037 per cent and the coefficient of variation was 4.5 per cent.

## CONCLUSIONS

1. A method is described for determining fat in skimmilk and nonfat dry milk solids which is suitable for routine use.
2. The method is based on measuring the area of a monolayer of fatty extract formed by transferring 0.007 to 0.008 ml. of its ether solution to a prepared surface on a solution of 0.2 per cent acetic acid.
3. Errors in the method are discussed.
4. Results are reported which indicate close agreement between the proposed method and standard Mojonnier procedures for the two products tested.

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# THE NUTRITIVE VALUE OF ALFALFA HAY. IV. BEET PULP, CORN GLUTEN MEAL AND SOYBEAN OIL MEAL AS SUPPLEMENTS TO AN ALL-ALFALFA HAY RATION FOR MILK PRODUCTION<sup>1</sup>

C. F. HUFFMAN AND C. W. DUNCAN

*Departments of Dairy and Agricultural Chemistry,  
Michigan Agricultural Experiment Station,  
East Lansing*

It generally is recognized that the total digestible nutrients (T.D.N.) that cattle receive from an all-roughage ration are not as efficient for milk production as when the roughage is supplemented with concentrates. Kellner and Köhler (14) attributed the lower milk-producing value of the T.D.N. in roughages to their high crude fiber content. In a previous report (11), it was shown that properly depleted cows failed to increase in milk production when corn starch or glucose was added to an all-alfalfa hay ration, but when an equal amount of corn or wheat replaced the starch, milk production always increased, even though the crude fiber content in the dry-matter of the ration was practically constant. These results signify that both corn and wheat supplied the unidentified factor(s) needed for more efficient milk production. Several investigators (3, 9, 10, 12, 13, 20) have reported that the replacement of part of the T.D.N. in roughage with various concentrates has increased milk production. Smith *et al.* (20) stated that the inadequacy of an all-alfalfa hay ration for milk production was not due to a deficiency of total protein. Huffman and Duncan (10) also showed that the lowered efficiency for milk production of cows depleted on an all-alfalfa hay ration was not due to a deficiency of cystine.

The possibility of a deficiency of dietary fat as being responsible for the inefficiency of milk production of the depleted cows was suggested by the low fat content of alfalfa hay. The work of Maynard *et al.* (16) also indicated that a certain level of fat was needed in the dairy ration for optimum milk production. The present paper reports the results obtained by replacing part of the T.D.N. in an all-hay ration with low-fat beet pulp, sugar beets, corn gluten meal and soybean oil meal.

The purpose of this investigation has been to elucidate the nature of the nutrient precursors which are necessary for efficient milk production but are lacking in an all-hay ration.

## EXPERIMENTAL

The method of handling the experimental cows and the depletion technique employed in this work have been described previously (9). Three Jersey cows (66, 77, 127) and five Holstein cows (D9, D14, C167, A6, A18) were used to study the effect of dry beet pulp supplementation. Two Holstein cows (D14, A6) were used to investigate the milk-stimulating power of sugar beets. Two Jersey

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TABLE 1  
Description of the hays, their chemical composition and their actual or calculated coefficients of digestibility

Trial no.	Cow no.	Moisture (%)	Ash (%)	Protein (%)	Ether ext. (%)	Crude fiber (%)	N.F.E. (%)	Dig. protein (%)	T.D.N. (%)	Description of the hays
1 <sup>a</sup>	D9	13.6	6.79	15.4 66	2.04 41	25.7 50	35.5 67	10.8	49.3	U.S. No. 2, No. 1 on leaf, 2nd cut. alf., 1937 crop
2	D14	14.6	5.97	14.5 66	1.33 41	28.2 50	35.4 67	9.6	48.6	Same as trial 1.
3	C167	16.6	5.79	12.4 72 <sup>b</sup>	2.19 34	29.8 43	33.2 71	8.9	47.0	U.S. No. 3, 1st cut. alf.-brome, 1938 crop
4	127 <sup>c</sup>	12.8	5.04	10.4 72 <sup>b</sup>	1.50 31	32.8 43	37.5 70	7.5	48.9	U.S. No. 2, 1st cut. alf.-clover-tim, 1943 crop
6	A64 <sup>d</sup>	11.7	6.75	16.4 71	1.67 0	29.0 41	34.5 69	11.6	47.3	U.S. No. 3, No. 1 on leaf, 2nd cut. alf., 1940 crop
9	D14 <sup>f</sup>	13.8	7.82	16.4 72 <sup>b</sup>	1.80 34	28.3 43	31.9 71	11.8	47.2	U.S. No. 1, 2nd cut. alf., 1937 crop
11	A6	10.6	6.67	10.9 67 <sup>b</sup>	0.88 18	35.5 45	35.5 64	7.3	46.4	U.S. No. 3, 1st cut. alf.-clover, 1937 crop
12	A15	12.7	6.09	13.1 67	2.12 37	30.5 57	35.5 62	8.8	49.9	Ungraded 2nd cut. alf.-brome, 1941 crop
13	74	11.9	6.37	9.4 67 <sup>b</sup>	1.98 18	39.0 45	31.4 64	6.3	44.8	U.S. No. 3, 1st cut. alf.-brome 1937 crop



TABLE 1 (continued)

Trial no.	Cow no.	Moisture (%)	Ash (%)	Protein (%)	Ether ext. (%)	Crude fiber (%)	N.F.E. (%)	Dig. protein (%)	T.D.N. (%)	Description of the hays
14	76	10.4	5.89	11.3 67 <sup>b</sup>	1.10 18	36.3 45	35.0 64	7.6	46.8	Ungraded 1st cut. alf.-clover-tim., 1942 crop
15	442 <sup>c</sup>	9.4	5.33	9.8 51 <sup>b</sup>	1.46 39	36.0 59	38.0 64	5.0	51.8	U.S. No. 3, 1st cut. alf.-brome, 1946 crop
16	A29 <sup>a</sup>	10.6	5.45	9.9 51 <sup>b</sup>	1.32 39	35.4 59	37.3 64	5.0	51.0	U.S. No. 3, 1st cut. alf.-brome, 1946 crop
18	D14	11.7	6.75	16.4 72 <sup>b</sup>	1.67 34	29.0 43	34.5 71	11.8	50.1	U.S. No. 1, 2nd cut. alf., 1939 crop
19	A6	14.6	5.97	14.5 72 <sup>b</sup>	1.33 34	28.2 43	35.4 71	10.4	48.7	U.S. No. 1, 2nd cut. alf., 1937 crop
20	78	11.6	7.19	13.3 72 <sup>b</sup>	2.30 34	30.4 43	35.2 71	9.6	49.4	U.S. No. 1, 1st cut. alf.-brome, 1943 crop

<sup>a</sup> The first line in each trial represents the chemical composition of the hay.

<sup>b</sup> The second line in each trial represents the coefficients of digestibility of the various hay fractions. Those marked with footnote b represent the calculated values, whereas all other values were obtained experimentally.

<sup>c</sup> Cow 66 (trial 5) received this hay.

<sup>d</sup> Cow 77 (trial 7) received this hay.

<sup>e</sup> Cow A18 (trial 8) received the same hay as cow D14 (trial 2).

<sup>f</sup> Cow A6 (trial 10) received this hay.

<sup>g</sup> Cow 423 (trial 21) received this hay.

<sup>h</sup> Cow A46 (trial 17) received this hay.

cows (74, 76) and six Holstein cows (A6, A15, A29, A46, D14, 442) were used to study the effects of corn gluten meal. One Jersey cow (78) and two Holstein cows (A6, 423) were used on the soybean oil meal experiment. Twenty-one individual trials are reported, but cows D14 and A6 were used on three and four different trials, respectively, with different hays (table 3). The cows varied from 43 to 466 days in lactation and from 0 to 172 days in gestation at the start of the experimental period. Non-pregnant cows were used in eight trials.

The milk was weighed at each milking and 3-day composite samples were taken for butterfat determinations. The equivalent number of pounds of 4 per cent fat-corrected milk (F.C.M.) were calculated by the formula proposed by Gaines (6). The cows were weighed at the same hour every third day. The average figures for each of the above measurements are recorded in table 3.

The length of time varied from 6 to 8 days prior to the addition of a supplement, depending on the length of time necessary to deplete the cow of the grain factor(s). The length of time the hay-supplement ration was fed varied from 9 to 36 days.

The 14 hays used in this study represent 8 crop years. Both first and second cuttings of hay harvested from early bloom to full bloom were used. In all cases the hays were fed within 1 yr. after harvest. The description of the hays, their chemical composition, coefficients of digestion, digestible crude protein and T.D.N. content are presented in table 1. The actual coefficients of digestion were used whenever they were available, otherwise the coefficients recommended by Morri-

TABLE 2

*The chemical composition, digestible protein and total digestible nutrient content of the supplements used in each trial*

Trial no.	Cow no.	Moisture	Ash	Protein	Ether ext.	Crude fiber	N.F.E.	Dig. protein	T.D.N.
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Beet pulp									
1	D9 <sup>a</sup>	11.6	2.78	8.94	0.10	19.8	56.8	4.2	67.3
3	C167	11.4	2.88	8.94	0.37	20.6	55.8	4.2	67.1
4	127 <sup>b</sup>	12.4	2.74	9.00	0.25	19.1	56.2	4.2	66.5
6	A6	13.5	3.89	9.56	0.79	19.3	53.0	4.5	64.0
Corn gluten meal									
11	A6	11.1	0.97	43.69	1.99	2.7	39.6	37.1	79.6
12	A15	10.0	1.73	42.06	1.58	2.3	42.3	35.8	79.8
13	74	9.2	1.51	45.31	2.39	3.7	37.9	38.5	80.9
14	76	9.5	1.51	47.06	2.12	1.4	38.4	40.0	81.0
15	442 <sup>c</sup>	10.0	3.00	43.47	3.02	3.0	37.5	36.9	79.6
18	D14	9.5	1.28	42.38	4.28	4.3	38.3	36.0	83.0
Soybean oil meal									
19	A6	10.8	5.87	45.63	0.71	5.4	31.6	42.0	77.5
20	78	9.5	5.67	46.75	2.37	5.3	30.4	43.0	78.7
21	423 <sup>d</sup>	10.7	5.55	44.70	4.04	6.1	28.9	41.1	77.9

<sup>a</sup> This beet pulp was used for trials 2, 7, 8 (Cows D14, 77, A18).

<sup>b</sup> This beet pulp was used for trial 5 (Cow 66).

<sup>c</sup> This corn gluten meal was used for trials 16 and 17 (Cows A29 and A46).

<sup>d</sup> This soybean oil meal was used in trial 15 (Cow 442), see table 3.

son (17) were used. The chemical composition, digestible protein and T.D.N. values for beet pulp, corn gluten meal and soybean oil meal, calculated from the more liberal recommendations suggested by Morrison (17), are presented in table 2. No chemical analyses were made on the sugar beets, but Morrison's digestible protein and T.D.N. values were used in the calculations in table 3.

### RESULTS

The data showing stage of lactation, body weight, butterfat test, average daily yield of 4 per cent F.C.M., hay and supplement intake, T.D.N. received and required and per cent of crude fiber ingested by each cow, calculated on the dry-matter basis, are presented in table 3. When dry beet pulp replaced part of the hay, on an equal T.D.N. basis, there was a marked reduction in crude protein intake, but when corn gluten meal or soybean oil meal replaced part of the T.D.N. in the hay, the crude protein intake increased markedly.

In trials 1, 3 and 6, the T.D.N. received per day during the hay periods and hay-beet pulp periods were essentially the same, while in trials 2 and 7, cows D14 and 77 received 2.6 and 2.4 lb., respectively, less T.D.N. per day than when fed hay alone. In trials 4, 5 and 8, the cows received about 1 lb. less T.D.N. during the hay-beet pulp periods than from the all-hay rations. The T.D.N. intake for the two cows receiving the sugar beet supplement was approximately equal, but there was a significant increase in milk production. In all but one of the eight trials with corn gluten meal, the nutrient intakes were about the same as during the hay periods. In trial 18, the T.D.N. intake was only 0.4 lb. more when 6 lb. of corn gluten meal replaced 6 lb. of corn starch. The lowest percentage increase in milk production was obtained from D14 (trial 18) which received over 4 lb. more T.D.N. during both supplemented periods than during the all-hay period. Less T.D.N. were fed in the three hay-soybean oil meal rations than in the hay rations.

A significant increase in F.C.M. was obtained in seven of the eight trials when part of the T.D.N. of hay was replaced by beet pulp. Cow A18 (trial 6) increased only 0.2 lb. per day in milk production but she had been milking for 465 days prior to the start of the experiment. It is likely that she was affected by some deficiency other than the grain factor(s), since a large excess of T.D.N. was received from both rations. The average increase in F.C.M. per cow per day obtained from the low-fat beet pulp supplement was 17.2 per cent (range, 1.2-34.2). Sugar beets stimulated decided increases in milk production in D14 and A6 (trials 9 and 10). Corn gluten meal produced a significant increase in milk production in all trials. In trial 11, 3 lb. of cottonseed meal replaced 3 lb. of corn gluten meal for a 12-day period and a drop in milk production resulted, but when the hay intake was reduced 8.8 lb. and 9 lb. of corn gluten meal replaced the 3 lb. of cottonseed meal, an increase of 7.9 lb. of milk per day was obtained for the next 15-day period. The T.D.N. intake was only 0.6 lb. more than was received from the all-hay ration. The feeding of 6 lb. of corn starch per day as a supplement to the all-hay ration (trial 18) depressed milk production, but when 6 lb. of corn gluten meal replaced the 6 lb. of starch, a marked increase in milk pro-

TABLE 3

*The effect of various supplements to an all-hay ration on the average daily yield of 4 per cent fat-corrected milk and on the crude fiber content of each ration, calculated on the dry-matter basis*

Trial no.	Cow no.	Exptl. In period milk		Body wt.	Fat test	F.C.M.		Feed intake		T.D.N.		Crude fiber in D. M.
		(d.)	(d.)			Yield	Incr.	Hay	Suppl.	Rec.	Req.	
		(d.)	(d.)	(lb.)	(%)	(lb.)	(%)	(lb.)	(lb.)	(lb.)	(lb.)	(%)
						Beet pulp						
1 <sup>a</sup>	D9	15	99	1192	3.43	28.4		38.5		19.0	18.9	29.7
		18	114	1162	3.42	33.8	19.0	23.6	10.5	18.7	19.2	27.5
2	D14	6	167	1240	3.20	19.8		44.3		21.5	15.5	33.0
		36	173	1209	3.21	23.2	17.2	24.3	10.5	18.9	16.7	29.7
3	C167	12	84	1264	3.03	18.5		33.8		15.9	15.5	35.7
		9	96	1250	2.90	22.3	20.5	24.0	7.0	16.0	16.7	32.8
4	127	15	224	740	5.66	6.6		20.0		9.8	8.0	37.6
		18	239	738	6.27	7.4	12.1	10.0	6.0	8.9	8.3	31.7
5	66	15	137	740	4.79	7.6		24.0		11.7	8.4	37.6
		15	152	738	5.36	10.2	34.2	14.0	6.0	10.8	9.2	32.9
6	A6	15	466	1237	2.87	16.7		38.6		18.3	14.7	32.8
		12	481	1228	3.23	16.9	1.2	24.9	10.0	18.2	14.8	29.9
7	77	15	335	841	6.04	12.4		29.1		13.8	11.0	32.8
		30	350	829	6.65	13.6	9.7	9.8	10.0	11.4	11.3	27.6
8	A18	15	283	1036	3.63	14.1		34.4		16.7	12.7	32.0
		15	298	1038	3.53	17.4	23.4	14.8	12.2	15.7	13.8	25.8
						Sugar beets						
9	D14	15	59	1238	3.14	28.3		45.0		21.5	18.4	
		15	74	1202	3.29	31.7	12.0	30.0	55.0	21.9	19.5	
10	A6	15	113	1037	2.69	24.8		42.0		20.1	16.2	
		12	128	1033	3.27	29.7	19.8	25.0	55.0	19.5	17.7	
						Corn gluten meal						
11	A6	12	158	1066	3.00	21.1		43.4		20.1	15.1	39.7
		12	170	1084	2.90	26.1	23.7	38.0	3.0	20.0	16.9	37.3
		12	182	1109	3.00	23.3		38.0	3.0 <sup>e</sup>	19.9	16.1	37.8
		15	197	1089	3.10	29.0	37.4 <sup>d</sup>	29.2	9.0	20.7	18.0	37.2
12	A15	15	261	1183	4.28	13.1		35.0		17.5	13.3	35.0
		21	276	1143	3.88	16.0	22.1	25.0	6.0	17.3	13.9	28.5
13	74	18	279	769	5.18	15.5		32.8		14.7	11.5	44.3
		24	297	758	5.37	19.0	22.6	14.0	6.0	11.1	11.7	32.0
14	76	15	248	869	4.35	8.9		24.8		11.6	10.0	40.5
		21	263	862	5.04	12.8	43.8	15.0	6.0	11.9	11.3	29.3
15	442	15	150	1119	3.60	20.7		50.0		25.9	15.3	39.7
		18	165	1117	3.40	24.7	19.3	43.0	3.5	25.1	16.6	37.0
		15	183	1105	3.30	24.8	19.8 <sup>d</sup>	43.0	3.5 <sup>e</sup>	25.1	16.5	37.3
16	A29	12	281	1181	3.40	9.8		32.8		16.7	12.2	39.6
		12	293	1175	3.40	12.5	27.6	26.8	3.0	16.1	13.1	35.9
17	A46	12	274	1136	3.90	9.1		35.8		18.3	11.7	39.6
		12	286	1145	4.00	10.5	15.4	30.0	3.0	17.7	12.3	36.3
18	D14	15	146	1233	3.36	26.0		44.3		22.2	17.7	32.8
		15	161	1212	3.14	24.4		42.1	6.0 <sup>f</sup>	26.2	17.1	29.2
		12	176	1229	3.23	26.4	8.2 <sup>e</sup>	43.1	6.0	26.6	18.8	29.3
						Soybean oil meal						
19	A6	15	234	1136	2.80	21.9		44.1		21.5	15.7	33.0
		21	239	1152	2.91	23.5	7.3	28.1	9.0	20.7	16.4	24.7
20	78	12	296	992	5.25	6.1		34.8		17.2	10.0	34.4
		30	308	966	5.00	9.2	50.8	20.0	6.0	14.6	10.1	27.7
21	423	15	43	1105	3.00	25.5		35.6		18.4	16.8	39.8
		18	58	1083	3.10	29.3	14.9	29.9	3.5	18.2	17.5	36.3

<sup>a</sup> The first line in each trial represents the all-alfalfa hay ration, whereas the second or more lines represent the hay-supplement ration.

<sup>b</sup> Supplement contained 9 lb. of beet pulp plus 3 lb. of corn sugar daily.

<sup>c</sup> Cottonseed meal.

<sup>d</sup> Percentage increase over the all-hay ration.

<sup>e</sup> Soybean oil meal.

<sup>f</sup> Corn starch.

<sup>g</sup> Percentage increase over the alfalfa-starch ration.

duction was obtained. The average increase in milk production was 24.0 per cent per cow per day (range, 8.2–43.8) when corn gluten meal supplemented the all-hay ration. Soybean oil meal also produced a significant increase in milk production on a lower T.D.N. intake. The average increase was 24.3 per cent for the three trials.

The per cent of butterfat increased in four trials, but was unchanged or slightly decreased in the other four trials when beet pulp supplemented the all-hay ration. The two cows fed the sugar beets showed an increase in per cent of fat but no significant differences were obtained when corn gluten meal or soybean oil meal were fed, except in trial 14. The corn gluten meal fed in trials 11 to 14, inclusive, and the soybean oil meal fed in trials 19 and 20 contained from 0.71 to 2.39 per cent ether extractive materials. The ether extract of the hays used in all of the trials varied from 0.88 to 2.30 per cent, but the increases obtained in milk production do not appear to follow the ether extract.

The last column in table 3 gives the percentage of crude fiber in each ration, calculated on the dry-matter basis. The lowest percentage increase in milk production (trial 19), other than trial 6, was associated with a 25.2 per cent reduction in crude fiber intake, whereas the lowest crude fiber reductions (trials 11 and 15) gave percentage increases in milk production of 23.7 and 19.8 per cent, respectively. The highest percentage increase in milk production was obtained in trial 20, when the crude fiber intake was 19.5 per cent less than that in the all-hay ration. It would appear from these data that a reduction in crude fiber intake is not necessarily correlated with a corresponding increase in milk production.

#### DISCUSSION

The replacement of part of the T.D.N. in the rations of properly depleted cows with dry sugar beet pulp resulted in an increase in the production of 4 per cent F.C.M. The largest increase in milk production was made by cow 66 (trial 5) when 6 lb. of beet pulp replaced 10 lb. of first-cutting alfalfa-clover-timothy hay. The smallest increase was made by cow A6 (trial 6) which had already completed 465 days of lactation at the beginning of the experiment. There is a possibility that some deficiency other than the grain factor(s) was responsible for this small increase, in view of the fact that an excess of 3.4 lb. of T.D.N. was consumed per day over that required. The increases obtained in F.C.M. are in agreement with the results secured by Smith *et al.* (20), who reported an increase in milk production in two cows when part of the T.D.N. in an all-alfalfa hay ration was replaced with beet pulp.

When 55 lb. of sugar beets replaced hay on an equal T.D.N. basis, two cows, D14 and A6 (trials 9 and 10), increased 12.0 and 19.8 per cent, respectively, in F.C.M. It would appear from the results obtained from supplementing the all-hay rations with low-fat beet pulp or sugar beets that the first deficiency of an all-hay ration is the grain factor(s) and not fat, as might be anticipated on the basis of the low fat content of alfalfa hay and the above supplements. Cow D14 (trial 2) was fed beet pulp for 36 days without being depleted of fat; however,

she had been depleted of the grain factor(s) for only 6 days prior to the replacement of hay with beet pulp. Maynard *et al.* (16) have reported that an optimum level of 4 per cent of fat in the grain mixture is necessary for efficient milk production.

Of the ten trials with beet pulp and sugar beets, the per cent of butterfat in the milk was significantly higher in five trials, approximately the same in three trials and only slightly lower in two trials. According to Byers *et al.* (2), a ration of alfalfa hay and ground soybeans containing 5.2 per cent dietary fat did not increase milk production when compared to a ration of alfalfa hay and soybean oil meal containing 2.7 per cent fat, but a significant increase was obtained in the per cent of butterfat when the high fat ration was fed. Gibson and Huffman (7) fed a ration composed of alfalfa hay, beet pulp, beet molasses and solvent-extracted soybean oil meal and obtained a pronounced increase in the per cent of fat in the milk when soybean oil replaced beet pulp on an equal T.D.N. basis, but the increase was of short duration and soon returned to normal. The cows became depleted in fat within 12 days on the low-fat basal ration, but the per cent of fat in the milk increased as the cows became depleted in fat or some factor associated with fat.

The results obtained with beet pulp and sugar beets indicate that the grain factor(s) present in grains and reported previously by Huffman and Duncan (11, 12, 13) and Smith *et al.* (20) is present also in root crops. Davis and Kemmerer (3) reported that milk production was increased when dry grapefruit peel was added to an all-alfalfa hay ration.

In view of the potency of low-protein grains, *i.e.*, wheat and corn, to supply the milk-stimulating factor(s) (11, 13), two high-protein concentrates, corn gluten meal and soybean oil meal, were used to replace part of the T.D.N. in the all-hay rations. Corn gluten meal was used in eight trials and soybean oil meal was used in three trials. Cow A6 (trial 6) was fed two levels of corn gluten meal. At the 3-lb. level, the F.C.M. increased 5 lb. per day, whereas at the 9-lb. level, the increase above the all-hay ration was 7.9 lb. These two feeding periods were separated by a 12-day period during which time 3 lb. of cottonseed meal were fed and a decline in milk production was observed. A direct comparison of corn gluten meal was made with soybean oil meal at the 3.5-lb. level (trial 15) and no significant difference in milk production was obtained from the use of these two supplements. This cow had a very good appetite for hay which accounts for the consumption of a large excess of T.D.N. over that required.

The increases obtained in milk production when low-fat corn gluten meal or soybean oil meal replaced part of the T.D.N. in the all-hay ration indicate further that fat *per se* is not the first deficiency encountered in feeding an all-hay ration.

The results secured from D14 (trial 18) show that the addition of 6 lb. of corn starch to an all-hay ration and the subsequent replacement of the starch with 6 lb. of corn gluten meal resulted in an increase of 2 lb. of F.C.M. above the hay-starch ration. The crude fiber was practically the same in both the hay-starch and hay-corn gluten meal rations. It has been shown previously (11) that milk production

increased when either corn or wheat replaced corn starch or corn sugar, on an equal T.D.N. basis, when properly depleted cows were used, but that the addition of starch or sugar also increased milk production when undepleted cows were used.

The possibility that the observed increase in milk production when grains, beet pulp or sugar beets replace part of the T.D.N. in the hay is due to increased digestibility appears to be unlikely in view of the results obtained by Watson *et al.* (21). They reported that the digestibility of barley by sheep and steers was the same whether fed in combination with timothy or alfalfa hay or alone. Burroughs *et al.* (1) showed that the digestibility of the dry matter in corn cobs was depressed by the addition of starch, but when the same amount of starch was added to alfalfa hay, a depression in digestibility of dry matter did not occur. They explained their results on the basis that alfalfa hay contained more of the essential nutrients for promoting the growth of rumen microorganisms than did corn cobs. The presence of unidentified factors in alfalfa, essential for efficient roughage digestion, has been postulated. Quin (18) found that the rate of fermentation of glucose in the rumen of sheep was slow when fed in combination with poor hay but when alfalfa hay replaced the poor hay the rate of fermentation of glucose increased markedly. The change in the rate of fermentation was correlated with the change in rumen flora. Similar results were reported by Elsdon (4). Louw *et al.* (15) showed that the addition of fresh alfalfa to a ration of poor hay supplemented with starch, casein, yeast and a mineral mixture overcame the depression of cellulose digestion. It is probable that when alfalfa hay is included in the ration, no decrease occurs in digestibility that can be attributed to disturbance of the normal rumen flora.

In six of the 21 trials, losses in body weight were greater than 15 lb., in two trials the gain in weight was more than 15 lb., but the cows in all of the rest of the trials showed weight losses or gains of less than 15 lb.

The depletion and partial T.D.N. replacement technique used in these studies resulted in a reduction in dry matter intake when concentrates replaced part of the hay. The effect of changing the dry matter intake on fill has been investigated by Ritzman and Benedict (19). The actual loss of fill in lactating dairy cows, fasted 4 days, was found to be about 15 per cent of the live weight, but the cows were fed 14 kg. of hay per day during the "so-called" fast. Hale *et al.* (8) reported that when a cow was changed from 30 lb. of alfalfa hay per day to 15 lb., there was a loss of 4.7 lb. in live weight per pound decrease in dry matter intake. When a cow was changed from 5 to 20 lb. and from 20 to 30 lb. of alfalfa hay daily, the weight increases per pound of dry matter intake were 5.6 and 9.9 lb., respectively. On this basis, a loss of 4.7 lb. of live weight per pound of reduced dry matter intake would account for the decline in body weight of all of the cows used in this study. Forbes *et al.* (5) studied the metabolism of a steer fed alfalfa hay alone, corn alone, and alfalfa hay and corn and found that live weight declined in proportion to the decrease in dry matter consumption. The loss was greater when the corn ration was fed and intermediate when hay and corn were

fed. The net energy was much greater per pound of dry matter, however, when both hay and corn were fed than when either was fed alone.

#### SUMMARY

Sixteen cows which had been depleted of the grain factor(s) were used in 21 trials to study the effect on milk production by replacing part of the T.D.N. in an all-hay ration with either beet pulp, sugar beets, corn gluten meal or soybean oil meal.

In 9 of 10 trials a significant increase in 4 per cent F.C.M. was obtained when various levels of beet pulp or sugar beets replaced an equal amount of T.D.N. in the hay.

In 9 of 11 trials a significant increase in F.C.M. was obtained when various amounts of corn gluten meal or soybean oil meal replaced an equal amount of T.D.N. in the hay.

The tendency for the per cent of butterfat in the milk to increase when beet pulp or sugar beets replaced part of the hay was observed.

In view of the low fat content of beet pulp, sugar beets and most of the corn gluten meals and soybean oil meals used in these trials, it is apparent that the first deficiency of an all-hay ration for milk production is not fat *per se*.

The results indicate that the "so-called" grain factor(s) or the unidentified milk-stimulating factor(s) is stored also in beet pulp, sugar beets, corn gluten meal and soybean oil meal.

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# PREDICTABILITY OF BREEDING EFFICIENCY IN DAIRY CATTLE<sup>1</sup>

DURWARD OLDS AND D. M. SEATH<sup>2</sup>

*Kentucky Agricultural Experiment Station, Lexington*

The rapid growth of the artificial insemination program and its general adoption by dairy farmers has greatly stimulated the interest in breeding efficiency. Some dairymen have herds requiring many repeat services. It is not unusual for such men to become discouraged and withdraw from the program for a year or more. Dairymen have been advised to cull their herds from the standpoint of breeding efficiency as well as type and milk production. In order to gain more specific information concerning sound advice to furnish dairymen who are posed with herd breeding problems or problems of culling, the present study was made.

Trimberger and Davis (6) reported on the predictability of breeding efficiency in the University of Nebraska dairy herd over an 8-yr. period of time. The number of services required by 133 virgin heifers had no value in predicting the number required for subsequent conceptions. In like manner, the number of services required for the previous pregnancy of 199 cows had no value in predicting the number of services required for the following pregnancy. They also stated that the same principle applies to herds. Included in their study was a comparison of the breeding efficiency of 92 dams with that of their daughters, from which they concluded it was not possible to predict the breeding efficiency of heifers from that of their dams.

In contrast to the results of the Nebraska study, Lasley and Bogart (3) reported the repeatability of breeding performance for 120 range cows. They found that 80 cows having a good breeding record the first year continued to have a good record the next 2 yr. The 40 cows which had a poor record the first year continued to have a poor record the next 2 yr.

Jones *et al.* (2), in reporting a study of the Oregon State College dairy herd covering a period of 25 yr. and including 368 cows of four breeds, concluded that the inheritance of low fertility and close-breeding practices might account for much of the infertility found in dairy herds today. Spielman and Jones (5) in a study of the same herd, found a correlation of  $0.546 \pm 0.118$  between the mean reproductive efficiency of foundation cows and that of their daughters.

Olds *et al.* (4) found no significant correlation between the breeding efficiency of 91 dams and that of their daughters in the Kentucky Agricultural Experiment Station herd.

## EXPERIMENTAL PROCEDURE

Herd record books for twenty local cooperatives of the Kentucky Artificial Breeding Association were obtained covering 2 yr. of operation (1947-48 and

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1948-49). Breeding efficiency data from these books were tabulated for 6,509 cows and 2,403 herds which were serviced both of the consecutive years. Breeding efficiency was based on "non-returns" with all cows having at least 4 mo. in which to return for another service. According to work by Barrett *et al.* (1), these non-returns should be a reasonable approximation of actual conception. For this reason, the term "conception" has been used frequently for simplicity instead of "non-returns."

#### RESULTS

There were 20,263 cows bred in the twenty locals during the first year of this study (1947-48), and of those only 6,509 or 32.1 per cent appeared in the records during the second year (1948-49).

It was found that 4,665 cows, each of which required only one service the first year, averaged 1.44 services the second; 1,372 cows requiring two services the first year averaged 1.54 services the second; 400 cows requiring three services the first year averaged 1.64 services the second. The 72 cows which required four services the first year averaged 1.65 services the second. As the number of

TABLE 1

*Per cent of cows which required 1, 2, 3 or 4 services the second year within groups conceiving at 1st, 2nd, 3rd or 4th service the first year*

No. of cows	Service at which probable conception occurred 1st yr.			
	1st	2nd	3rd	4th
	4665	1372	400	72
		% of cows		
Services required 2nd yr.				
1	68.5	63.0	57.3	62.5
2	21.0	23.3	24.8	16.7
3	8.4	10.3	14.0	13.9
4	2.1	3.4	4.0	6.9

services required the first year increased, there was a rather uniform increase in the average services required the second year. However, the differences were not great, averaging 0.1 service between the first two groups and less than that between the third and fourth service groups (0.01 service). The number of cows for the fourth group (72) was markedly smaller than for the other groups.

The number of cows conceiving at first, second, third or fourth service the first year and the percentage of each group which required one, two, three or four services the following year are given in table 1. As the number of services required the first year increased, the per cent of cows conceiving at first service the following year showed a downward trend, except in the last or fourth service group. The irregular trend for this group may be due to the smaller number of cows (72) involved. As the number of services required the first year increased, the per cent of cows conceiving at second and third service the following year showed a highly significant upward trend. Again, the small fourth service group did not follow this trend.

The correlation between the number of services required by cows the first year as compared to the number of services for the second year was  $0.084 \pm 0.012$  ( $p < .01$ ). This correlation, although statistically highly significant, was too small to indicate a high degree of predictability. The coefficient of determination ( $r^2$ ) shows that only 0.71 per cent of the variation between cows the second year could be accounted for by their performance the first year.

The percentage of cows which required the same number of services both years was 54.9. As would be expected, those requiring but one service the first year scored highest in this regard, with 68.4 per cent of them also having but one service the second year. In contrast, only 23.3 per cent in the two-service group, 14.0 per cent in the three-service group, and 6.9 per cent in the four-service group had identical records for the second year.

*Predictability of breeding efficiency for herds.* There were 2,403 herds in which cows were bred during both of the 2 yr. The average herd consisted of 7.1 cows the first year and 8.3 cows the second. The breeding efficiency was based upon average number of services per cow, using the herd as a unit.

Table 2 shows the number of herds within each of four efficiency groups and

TABLE 2  
*Repeatability of breeding efficiency of herds*

No. of herds	Av. no. of services required the 1st yr.			
	1.0-1.5	1.6-2.0	2.1-2.5	2.6-3.0
	1526	705	121	51
Services required 2nd yr.	% of herds			
	1.0-1.5	1.6-2.0	2.1-2.5	2.6-3.0
1.0-1.5	69.0	59.6	50.4	70.6
1.6-2.0	25.6	34.3	36.4	29.4
2.1-2.5	3.5	3.8	8.3	0.0
2.6-3.0	1.9	2.2	5.0	0.0

the per cent of these herds which were in each of these groups the second year.

The percentages for the various groups and the trends shown in table 2 are somewhat similar to those of table 1. It will be of some encouragement to dairy-men with problem herds to notice that of the 172 herds which averaged more than two services per cow the first year, only 16 herds (9.3 per cent) were still that low the next year. Also, it is of interest that 54.3 per cent of the herds had about the same breeding efficiency both years. This figure was determined largely from data of herds averaging less than two services per cow. The portion of the total herds that were "problem herds" (having 2.1 or more services per cow) remained about the same both years, i.e., 7.1 per cent the first year and 5.9 per cent the second.

#### SUMMARY

The breeding efficiency of 6,509 cows and for 2,403 herds was compared for 2 consecutive yr. As the number of services required by cows the first year in-

creased, there was a rather uniform increase in the average number of services required the second year. However, the differences were not great, increasing approximately 0.1 service for each service increase of 1.0 for the first year. Nearly 55 per cent of the cows required the same number of services both years. The correlation between breeding efficiency for consecutive years was  $0.084 \pm 0.012$ .

The predictability of breeding efficiency of herds was about the same as that for cows. About 54 per cent of the herds required approximately the same average number of services per cow both years. Only 9.3 per cent of the "problem herds" (averaging 2.1 or more services per cow) were still problem herds the next year. The total number of problem herds remained about the same both years, *i.e.*, 7.1 per cent the first year and 5.9 per cent the second.

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## STUDIES ON GROWTH AND SURVIVAL OF CALVES FED SEMI-SYNTHETIC MILKS FROM BIRTH<sup>1</sup>

JOE KASTELIC, ORVILLE G. BENTLEY AND PAUL H. PHILLIPS

*Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison*

The use of purified diets to study the nutritional needs of young calves is a relatively recent development. Johnson *et al.* (11) reported subnormal growth of colostrum-fed calves raised on a purified mixture of casein, lactalbumin, sugar, lard or butter and water. Supplementation with riboflavin, thiamine, yeast or grass juice did not give consistent improvement in growth. Wiese *et al.* (17) reported the development of a synthetic milk which promoted good growth in young calves. They observed that growth rates of calves receiving the synthetic milk with lard were normal according to the Ragsdale standard (15), but that calves fed this milk with soybean oil grew poorly. In an experiment reported by Flipse *et al.* (6) on three 5-day-old calves, corn sugar was found to be superior to dextrin or corn starch when these carbohydrates were incorporated into milk substitutes. Investigations by Gullickson *et al.* (7), Bate *et al.* (2) and Jacobson *et al.* (10) have shown that dairy calves do not grow well and are subject to digestive disturbances on diets containing oils from plant sources fed with skim milk.

While it generally is believed that colostrum is indispensable to the newborn calf, studies by Lundquist and Phillips (13) and Hansen *et al.* (8) have demonstrated that Holstein calves could be raised on a diet composed solely of skim-milk and added vitamins.

These successes prompted a study to determine whether the young calf could be raised on a synthetic diet without either milk or colostrum. For this purpose a semi-synthetic milk was developed to replace the normal colostrum and whole milk diet. The effect of variations in the composition of this milk on the survival and growth performance of young calves was studied.

### EXPERIMENTAL PROCEDURE

The calves used in the experiments were obtained from local farms and from the station dairy herd. These calves were denied colostrum by prearrangement with the owners of the dams. Since the animals were not protected by colostrum feeding, experiments were conducted in the laboratory animal room except when otherwise noted. Weights of the animals were obtained when received and at approximately weekly intervals while on the experimental rations. Protocols were kept on each calf. Blood samples for serum nitrogen and for electrophoretic studies were taken from a few of the animals before they were given food and at various intervals thereafter. Post-mortem examinations were made.

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Antibiotics were used as indicated since experience had shown that colostrum-free calves often succumb to infections which are harmless to older animals.

Two semi-synthetic milks were used. Milk 1 (Ca: Na of 0.7: 0.9) (table 1)

TABLE 1  
*Composition of synthetic milks 1 and 2*  
(g. per kg. final liquid milk)

Components milk 1		Components milk 2	
Crude casein	35.0	Crude casein	35.0
Cerelose	50.0	Cerelose	50.0
Cottonseed oil	10.0	Butterfat <sup>c</sup>	" "
Egg albumin	8.0	Salts <sup>d</sup>	" "
Salts IV <sup>a</sup>	4.0	Citrus pectin	2.0
Soya lecithin <sup>b</sup>	2.0	Soya lecithin	2.0

<sup>a</sup> Salt mixture of Phillips, P. H., and Hart, E. B. J. Biol. Chem., 109: 657. 1935.

<sup>b</sup> Generously supplied by Associated Concentrates, Inc., 32nd Ave., Woodside Long Island, N. Y.

<sup>c</sup> When fed, the level was 2.5 or 3.5%.

<sup>d</sup> See table two.

was prepared as described by Wiese *et al.* (17) with some modifications. A water emulsion of soya lecithin and cotton seed oil prepared in a Waring blender was added after the casein, egg albumin, sugar and salts were brought into solution. Water was added to give a solids content of 109 g. per liter. The emulsification of the oil was considered advisable since it had been shown by Gullickson *et al.* (7) that a diet of lard homogenized into skimmilk was satisfactory for calves. The fact that vegetable oils were not tolerated despite homogenization presented the possibility that they contained insufficient phospholipid. The artificial milk was fed at a temperature of 37° C. from nipple-fitted pails. Calves were fed twice a day, half the daily allowance at each feeding. All the known vitamins except B<sub>12</sub> were fed in generous amounts, as indicated in table 2.

TABLE 2  
*Vitamin supplement*

Vitamin	Quantity
Ascorbic acid	150 mg. capsule/d.
Vitamin A	250,000 I.U. capsule first 3 d.
"    A	5,000 I.U. " daily from 4th d.
"    D	500 I.U. " " " " "
Thiamine	5.0 mg.
Riboflavin	5.0 "
Pyridoxine	5.0 "
Ca Pantothenate	7.5 "
Niacin	15.0 "
Inositol	100.0 "
Menadione	1.25 mg.
Biotin	0.05 "
Pteroylglutamic acid	0.25 "
p-Aminobenzoic acid	12.50 "
Choline	1.0 g. mixed into milk daily
α-D-tocopherol	40.0 mg. " " " "
B <sub>12</sub> <sup>a</sup>	7-10 γ/d.

<sup>a</sup> Estimated from analysis of crude casein.

## RESULTS

Three calves were fed (per 100 lb. of live weight) daily levels of 3 to 7 kg. of synthetic milk 1. The data in table 3 show the results of this experiment, which

TABLE 3  
*Summary data on new-born calves fed synthetic milk 1*

Calf	1	2	3
Breed	Ayrshire	Brown Swiss	Holstein
Birth weight	85 lb.	93 lb.	93 lb.
Time diarrhea developed	24 hr.	24 hr.	24 hr.
Preventive measures	fat removed	no fat fed	no fat fed
Duration of diarrhea	5th d.		
Apparent cause of death	?	3 d. pneumonia	2 d. pneumonia

was conducted in an isolated area of the Station dairy barn. In this experiment all attempts to control diarrhea were unsuccessful. Reduced feedings, administration of Kaopectate and the removal of the fat from the ration were without beneficial effect. It was impossible to determine the causes of the diarrhea. Antibiotics were not used. Post-mortem examination revealed a general gastro-enteritis. There was no evidence of coagulated casein in the stomachs, and the ingesta of the intestinal tract was liquid, resembling the synthetic milk in appearance. Pneumonia was the terminal cause of death of the two animals. Since there was no indication of digestion in the gastro-intestinal tract, evidence was sought that would throw light on this problem.

It has been shown repeatedly that rennet coagulation of milk is inhibited in the absence of sufficient amounts of calcium and retarded by improper ionic balance. Other methods of preparing a synthetic milk then were investigated. A casein solution containing salts in concentrations which occur in normal milk was prepared according to the method of Clark (4). Lime water was used in place of the  $\text{NaHCO}_3$  solution to dissolve the casein. The remaining elements were added according to his method with the exception that 0.1 *N* HCl was used to adjust the final pH of the milk to 6.6. To this casein solution were added cerelese, soya lecithin and trace minerals to give milk 2 (Ca:Na of 2.5:1.0) with the final composition shown in tables 1 and 4. When fat was added, it was

TABLE 4  
*Salt composition of synthetic milk 2<sup>a</sup>*

Component	Quantity/kg. milk	Component	Quantity/kg. milk
$\text{Ca(OH)}_2$	1.200 g.	KOH	0.959 g.
MgO	0.298 "	NaOH	0.928 "
$\text{KH}_2\text{PO}_4$	2.873 "	Fe citrate	2.0 mg.
Citric acid	1.998 "	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.1 "
$\text{CaCO}_3$	0.140 "	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.5 "
HCl (0.1N)		$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	10.0 "
$\text{CaCl}_2$	1.565 g.	$\text{CoSO}_4 \cdot 5\text{H}_2\text{O}$	1.5 "
		KI	0.4 "

<sup>a</sup> M. W. Clark. Synthetic milk as a basis of research. J. Dairy Sci., 10: 195. 1927.



emulsified with an aqueous suspension of soya lecithin before addition to the milk. Some difficulties were encountered in the initial attempts to make a stable casein solution. Some of the dissolved casein often would precipitate out as small curdy masses when the solution of  $MgO$ , citric acid and  $KH_2PO_4$  was added. Efforts to correct this difficulty were made by adding pectin to the casein solution before adding the phosphate solution. The pectin greatly improved the stability of the casein solution in the milk. However, in later work and after more experience had been gained in the preparation of the milk, it was found that precipitation of the casein could be avoided if the phosphate solution was slowly sprayed into the rapidly mixing casein solution. A few calves fed the synthetic milk containing the pectin also received milk without it but no difference in response was noted, hence its use was discontinued.

A considerable number of tests on the behavior of this milk towards rennet coagulation were made. In almost every instance the milk was coagulated in from 3 to 5 min. after the addition of rennet. Comparative tests showed no coagulation of artificial milk 1 (the low Ca milk) during holding periods as long as 3 hr. The data obtained with six calves which were fed synthetic milk 2 are summarized in figure 1.

Calf 4 was kept in the dairy barn, while the other animals were kept in the laboratory animal room. The first experiments were made with milk 2 without fat. Calves 4 and 5 died within 4 and 6 days, respectively, from *Escherichia coli* infections. There was no evidence of diarrhea in these calves and post-mortem examination revealed large clots of casein in their stomachs, a condition not observed in the animals which failed on milk 1. After discovery that the *E. coli* infection had caused the deaths of the animals, the antibiotic dihydrostreptomycin was used to prevent further losses from this cause.

In subsequent experiments, administration of streptomycin was resorted to in all animals which developed a rise in temperature and a rapid rate of respiration. Only one death (calf 8) occurred among the animals used in the remaining series of investigations. This animal refused food upon arrival and could not be induced to take more than a small amount of food.

A crisis period was observed in about 90 per cent of the calves. This critical period occurred chiefly during the second and third day of life. Once this period was successfully negotiated, an increase in vitality was noted. In the presence of a proper diet, an immediate increase in food intake occurred with a corresponding increase in body weight.

Calves 6, 7 and 9 were maintained on the fat-free synthetic milk 2 for 18, 14 and 6 days, respectively. Except for calf 9, which refused to take more than one-half of the allotted milk during the first week, these animals made gains comparable to the Ragsdale standard (14). In order to test the feeding of fat with phospholipid, these three calves were given 2.5 per cent butter oil, emulsified in the presence of soya lecithin. The abrupt change to fat-containing diet did not cause digestive disturbances in any of the calves nor was any difficulty encountered when they were transferred to whole milk at the end of the experimental period.

Further studies of the effect of phospholipid upon fat utilization were made. Butter oil (3.5 per cent) and soya lecithin were emulsified and fed to calves 10, 11 and 12. These animals responded to the ration much better than the non-fat

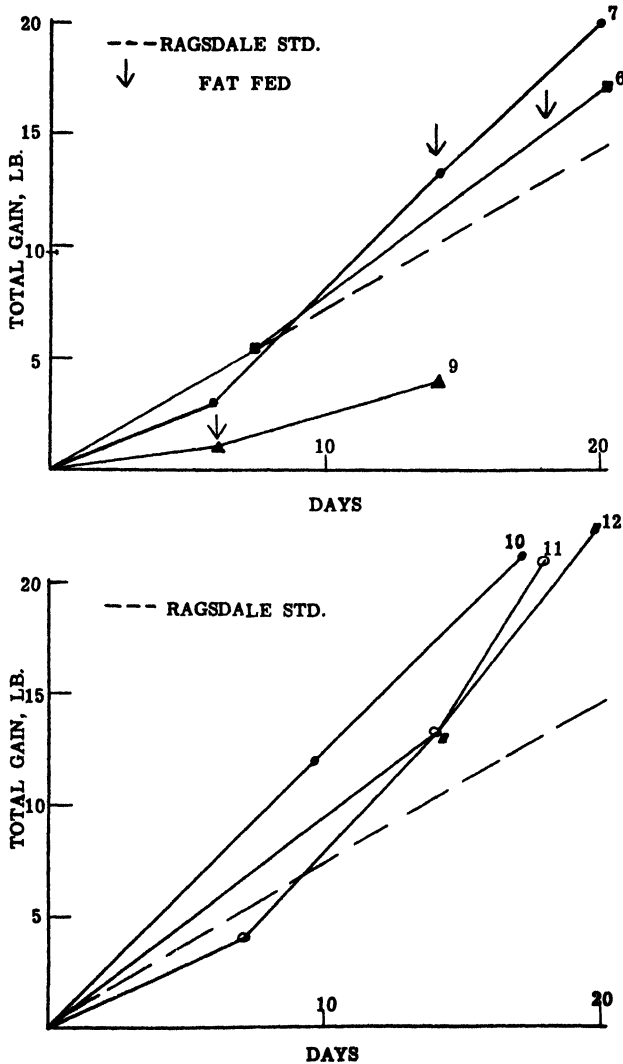


FIG. 1. Growth performance of colostrum-free Holstein calves fed synthetic milk 2. Weight gains in top figure are shown for calves not fed fat until the day indicated by arrow. Lower figure shows growth performance of calves fed milk 2 with butterfat from birth.

control group and made gains considerably higher than the Ragsdale standard (figure 1). Scouring did not develop in any of the calves. They were apparently normal animals.

Blood protein studies made on calves 6, 7, 11 and 12 revealed slight abnormalities. At approximately 2 wk. of age, these animals had serum protein concentrations of 5.0, 5.5, 5.6 and 5.0 g. per cent, respectively, values which appear to be slightly lower than those observed in colostrum and milk-fed calves of similar age. Electrophoretic data summarized in table 5 indicate that the

TABLE 5  
*Comparison of the distribution of blood serum proteins of calves fed synthetic milk 2 and skim milk*

Age		% of total blood serum protein			
		albumin	$\alpha$ globulin	$\beta$ globulin	$\gamma$ globulin
2 wk.	synthetic milk 2	41.8	30.6	26.8	7.2
Birth		45.2	42.5	9.3	2.5
2 wk.	skim milk*	47.0	30.2	17.9	4.8

\* Data, Hansen and Phillips (8).

ration gave results similar to those found by Hansen and Phillips (9) in skim-milk-fed calves denied colostrum. Otherwise all other observations indicated that the blood constituents were essentially normal. Blood vitamin A was checked in calf 6 at 14 days of age and found to be 22  $\gamma$  per 100 ml. of serum.

The excellent performance and gains made by the calves on milk 2 led to further investigations on the importance of rennet coagulation of the milk and of the essentiality of soya lecithin in the utilization of the fat. A diet which was a modification of milk 2 was used in these studies. A series of milks containing various levels and ratios of calcium and sodium was prepared in the same way as milk 2.  $\text{NaHCO}_3$  was added as a solution to casein suspended in varying amounts of lime water to give milks containing concentrations of sodium from 0.63 to 0.98 g. and levels of calcium from 0.80 to 1.20 g. per liter of synthetic milk. As in the case of milk 2, the pH was adjusted to 6.6. Coagulation times for these milks are shown in table 6 when a 1:50 dilution of

TABLE 6  
*The effect of variation in levels of Ca and Na in synthetic milk on coagulation by rennet*

Milk	Ca g/l.	Na g/l.	Coagulation* at 40° C.
milk 1	0.73	0.87	none
milk 2	1.26	0.53	4 min.
test milk	0.80	0.98	none in 2 hr.
" "	0.90	0.98	" " "
" "	1.00	0.98	" " "
" "	1.10	0.98	some in 30 min.
" "	1.20	1.18	3 min.
" "	1.20	1.28	4 "
" "	1.20	1.38	3 "
" "	1.30	1.38	3 "

\* Commercial rennet at 1:50 dilution.

commercial rennet was added. A calcium content of at least 1.10 g. per liter was necessary before coagulation occurred. A milk similar in composition

to milk 2, except for the calcium and sodium ratio, was selected for study. It contained 0.80 g. of calcium and 0.98 g. of sodium and was designated as milk A. This milk closely approximated milk 1 with respect to the Ca:Na ratio and ion concentrations and, like milk 1, was not coagulated by rennet.

Milk 2 was fed to another group of calves to determine whether cottonseed oil emulsified with soya lecithin would be tolerated similarly to the emulsified butter oil. First, a Guernsey calf (no. 13) was fed milk 2 with cottonseed oil emulsified in a Waring blender with soya lecithin and water. After 2 days the calf began to show definite indications of diarrhea. Microscopic inspection of the emulsion revealed considerable numbers of large oil globules which were not completely emulsified by the Waring blender. In order to further reduce the size of the fat globules in the ration of the calf, a mixture of 10 lb. of cottonseed oil in 30 lb. of water containing 400 g. of soya lecithin was emulsified by means of a Gaulin single-action homogenizer at 1800 lb. pressure. The oil-water emulsion was re-cycled through the homogenizer until microscopic examination showed that the oil globules were  $2\ \mu$  or less in diameter. When 2.5 per cent cottonseed oil prepared in this manner was added to milk 2 and fed to the calf (now 12 days old), the diarrhea was stopped within 36 hr. On the basis of this evidence all other milks containing cottonseed oil were Gaulin-homogenized.

The growth performance of calves 16, 17, 19, 20 and 22 on milk A (Ca: Na of 0.8:1.0) and that of calves 13, 18 and 21 which were fed milk 2 (Ca:Na of 2.5:1) are shown graphically in figure 2. Two and a half per cent cottonseed oil was included in both milks. The calves fed the low Ca milk lost considerable weight during the first week and only two animals survived. Severe diarrhea was observed beginning at 24 hr. of age and after the animals had received two feedings of this diet. Their appetites remained good for a few days and they consumed all their milk. Calves 16, 17 and 22, however, continued to scour severely, became weak and listless, finally refused food and died a few hours later. Calves 19 and 20 began to rally at 8 to 10 days of age and to gain weight coincident with a decrease in severity of the diarrhea. The condition of these calves was poor, but they were alert and had good appetites.

The response of calves on milk 2 (Ca: Na of 2.5: 1.0) with cottonseed oil was good. However, calf 21 made slow gains. This calf was very weak at birth. Its knee joints were enlarged and rachitic and there was a large boney growth on its lower jaw. The animal had a poor appetite for the first week and scoured on the seventh and eighth days. It was constipated for the following 4 days, but a marked improvement in appetite and gain in weight were observed following this period. When it was taken off the experiment on the 24th day, the bone abnormality had disappeared and general appearance of the calf was good. Calf 13, after the change to the thoroughly homogenized oil, made rapid gains, did not scour again and appeared normal in every respect. Calf 18 made gains which paralleled the Ragsdale standard throughout the period.

The apparent importance of feeding the very young calf a milk which would coagulate when rennet was added prompted other experiments. Two calves, a

Guernsey and a Holstein, were used in a reversal experiment. Figure 3 shows the growth of the calves used in these studies.

Calf 14 was fed milk A (Ca:Na of 0.8:1.0) with cottonseed oil from birth to 8 days of age. Its reaction to the ration was identical to that observed in other animals except that it had less appetite. On the eighth day the ration

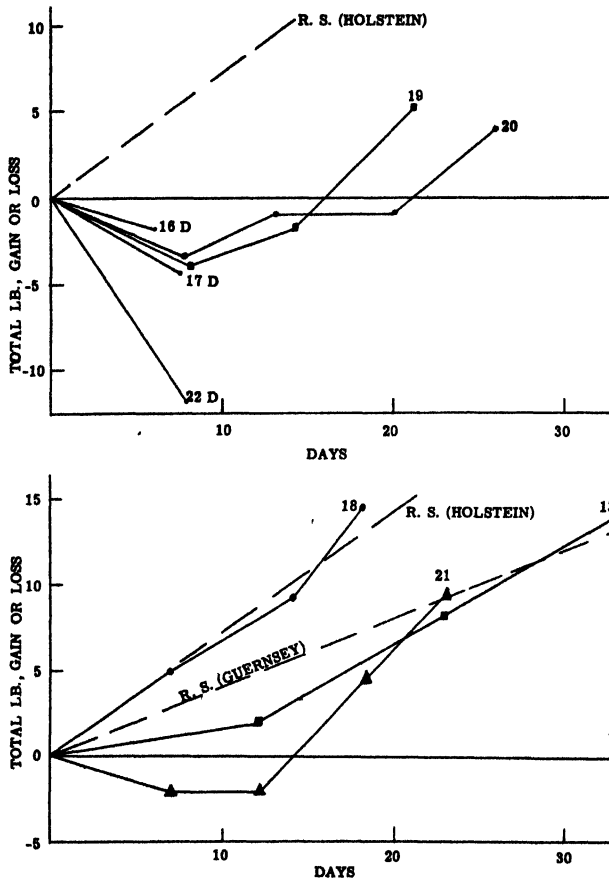


FIG. 2. Growth performance of colostrum-free calves fed synthetic milk with 2.5% cottonseed oil. Upper figure shows growth data of calves fed milk A. (Sodium and calcium concentration in this milk was altered to prevent rennet coagulation.) Lower figure shows growth response of calves to milk 2 with 2.5% cottonseed oil.

Calves number 13 and 16 were Guernsey, calf 19 Ayrshire and the remaining animals Holsteins. Legend: R. S., Ragsdale standard; D, died.

was changed to milk 2 (Ca:Na of 2.5:1) with cottonseed oil. The scouring soon stopped and rapid gains in weight followed. The Guernsey (15) was raised to 14 days of age on milk 2 and butter fat before being placed on the milk A with cottonseed oil. Indigestion and scouring did not appear following the change

in diet and the animal continued to make excellent gains in weight. Figure 3 shows that this animal made gains in weight which were much higher than expected on the basis of the Ragsdale standard for Guernseys.

#### DISCUSSION

From the results of these experiments it is evident that the synthetic milk 2 (Ca: Na of 2.5:10) was a satisfactory diet for colostrum-free newborn calves. Calves fed milk 2 grew normally and did not develop diarrhea. Milk A, which was similar in composition to milk 2 except that ratio and concentration of calcium and sodium were altered sufficiently to prevent coagulation by rennet, was not tolerated by newborn calves. Calves fed this milk, without exception, developed severe diarrhea, became unthrifty, and three of five animals died

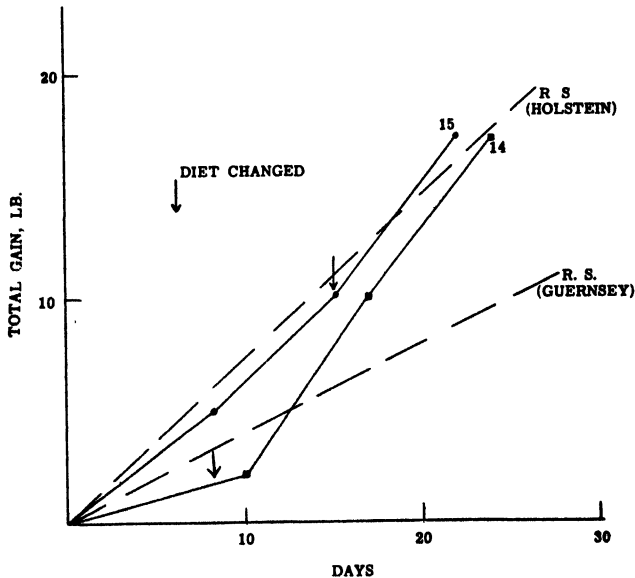


FIG. 3. Weight gains of calf 15 (Guernsey) fed milk 2 with 2.5% butter fat to 14 d. of age then changed to a diet of milk A with 2.5% cottonseed oil. Calf 14 (Holstein) received milk A with 2.5% cottonseed oil for the first 8 d. of life, and then was fed milk 2 with 2.5% cottonseed oil for the remainder of the experimental period.

within a week. However, it was found that a diet of milk A was satisfactory for the 2-wk.-old calf. These facts and the observation that newborn calves which survived on milk A exhibited a gradual decrease in severity of diarrhea and progressive improvement in weight gains with increasing age, clearly indicate that rennet coagulation of milk is essential for the well being of calves during the first days of life. The satisfactory performance of older animals fed milk A can be explained by assuming that the secretion of acid and pepsin in the abomasum was sufficient to cause coagulation of the ingested milk in the older calf.

These considerations are in harmony with other observations and facts. According to Best and Taylor (3), rennin is especially abundant in the gastric mucosa of young animals and pepsin is present in minimal amounts. Since rennin activity is completely abolished in the presence of pepsin at a low pH, one must conclude that rennet coagulation of milk in the stomachs of animals occurs only in a moderately acid environment. On the other hand, pepsin would be inactive in the optimum pH range observed for rennin activity, *viz.* pH 6.0-6.6. That rennin activity cannot be wholly responsible for the coagulation of milk in older calves may be concluded from observations made by Mortenson *et al.* (14). Their investigations on fistulated calves revealed that the hydrogen ion concentrations of stomach contents during curd digestion varied between pH 2 and 3.

With these facts in mind, it is to be concluded that coagulation of milk in the stomachs of calves is of vital importance to the proper digestion and assimilation of this food. This would insure retention of milk in the abomasum and allow time for partial digestion of the casein, globulin and albumin before passage into the intestinal tract. Aside from this consideration it is apparent that the rate of food passage through the gastro-intestinal tract would be markedly influenced by the physical condition of the milk constituents. Semi-solid substances, such as coagulated milk, would not move down the tract as rapidly as liquids. The marked improvement in response of calves to rennet incoagulable milk as they grew older, coincident with an increased development of gastric secretion, is additional evidence that coagulation of ingested milk is an essential step in the assimilation of this food during early life. These facts offer evidence for the delayed development of the pepsin-HCl digestive functions in the calf. Apparently, the newborn calf is wholly dependent on rennin action as a digestive aid until the pepsin-HCl function is developed.

The response of calves to butter oil and cottonseed oil revealed few, if any, differences. It was demonstrated that it was necessary to thoroughly homogenize the cottonseed oil in the presence of soya lecithin to avoid digestive disturbances. However, partially emulsified butter oil was found to be satisfactory.

An explanation for this difference is not readily apparent. While the presence of soya lecithin was essential for the production and subsequent stabilization of the finely divided oil globules in an aqueous medium, the soya lecithin may have made other contributions. It is reasonable to assume that the great increase in the surface area of the oil resulting from the reduction of the oil globules to microscopic dimensions would increase the rate of lipase action and subsequent assimilation of this oil. A study by Augur *et al.* (1) showed that the addition of lecithin to the diet improved the digestibility of cottonseed oil or hydrogenated cottonseed oil in the rat. Diarrhea occurred less frequently among animals fed high fat and lecithin than in the control high-fat group. The beneficial effect of lecithin on fat digestion was explained by suggesting that lecithin increased the rate and degree of fat emulsification. The effect of emulsifying agents on fat utilization by human subjects with nutritional difficulties was studied by Jones *et al.* (12). The marked reduction of stool fats when

Tween 80 was included in the diet is further evidence that surface active agents play an important role in fat utilization. The observations reported by Shantz *et al.* (16) that rats had better hair coats and appeared thriftier when egg lecithin was fed with corn oil and coconut oil also suggest that lecithin enhances fat utilization. Further evidence that lecithin is important in nutrition was indicated by Esh *et al.* (5). They found that addition of lecithin to skimmilk increased the vitamin A blood level in young calves. The fact that Gullickson *et al.* (7) found that vegetable oils homogenized into skimmilk did not make a satisfactory diet for calves may be due to the lack of sufficient amounts of lecithin in skimmilk. The satisfactory response of calves to the cottonseed oil when thoroughly homogenized in the presence of soya lecithin seems to bear this out.

#### SUMMARY

A semi-synthetic milk coagulable by rennet was developed and found to be nutritionally adequate for colostrum-free calves when streptomycin was administered.

Fat could be fed with this milk without adverse effect. Butter oil emulsified with soya lecithin in a Waring blender gave better growth results than cottonseed oil similarly dispersed. However, an excellent response to cottonseed oil was observed when the oil was thoroughly homogenized with an aqueous suspension of soya lecithin before addition to the milk.

The distribution of blood serum proteins of animals fed the rennet coagulable synthetic milk was similar to that observed in colostrum-free calves fed skimmilk.

A synthetic milk in which the calcium and sodium balance was altered to prevent rennet action produced severe diarrhea among newborn calves. This milk was a satisfactory diet for older animals. This fact offers evidence for a delayed development of pepsin-HCl digestion function in the calf.

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## FERTILITY OF DILUTED BULL SEMEN CONTAINING 100 MICROGRAMS OF STREPTOMYCIN PER MILLILITER<sup>1</sup>

H. L. EASTERBROOKS, P. HELLER<sup>2</sup>, W. N. PLASTRIDGE,  
E. L. JUNGHERR AND F. I. ELLIOTT

*Storrs (Connecticut) Agricultural Experiment Station, University of Connecticut, Storrs*

The recent discovery of antibiotics has aroused interest concerning their possible use in increasing fertility of diluted bull semen. In 1947, Salisbury and Knodt (8) reported that the addition of sulfanilamide produced increased fertility rates. Almquist (1, 2, 3) has shown that both penicillin and streptomycin increase the fertility rates of certain relatively infertile bulls and with his co-workers (4) has reported that levels of streptomycin above 100  $\gamma$  per milliliter of diluted semen were especially effective in controlling bacteria. They also showed that levels of streptomycin up to 1,000  $\gamma$  per milliliter did not significantly decrease sperm livability. It was the objective of this study to test the addition of streptomycin to diluted semen used in routine artificial breeding.

### EXPERIMENTAL

All 16 bulls in use at the time by the Connecticut Artificial Breeding Association were subjected to the conditions of the experiment. All 84 ejaculates collected and used during the first 4 mo. of 1949 were utilized in a split-sample type of study.

Semen, after routine collection and examination, was diluted at an average ratio of 1 to 75 in a diluter composed of equal parts egg yolk and sodium citrate (3.6 per cent  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 5\frac{1}{2}\text{H}_2\text{O}$ ) plus sulfanilamide (0.6 per cent) in sterile distilled water. The diluted semen then was divided into two portions. The first portion served as a control, while to the second portion, 100  $\gamma$  of streptomycin sulfate were added to each milliliter.

Inseminators were divided into two groups so that each group would inseminate a similar number of cows over a given period of time. Each inseminator group received alternately treated and non-treated samples from each bull.

The per cent of 60- to 90-day non-returns (N. R.) to first service was utilized as the basis of comparison. Consideration was given only to first services in accumulating these data.

### RESULTS

A total of 4,719 first services were recorded by the Association during the course of this study. Of these, 2,340 cows were inseminated with control semen and 2,379 with semen containing streptomycin.

When the operation was considered as a whole, the 60- to 90-day N. R. per cent for the control group was 61.1 and for the streptomycin-treated group, 69.8.

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<sup>2</sup> Manager, Connecticut Artificial Breeding Association

This was an increase of 8.7 N. R. per cent for the 4-mo. period for streptomycin-treated samples.

Differences in N. R. per cent, when converted to angles and weighted as described by Landauer and Bliss (6), showed a highly significant ( $P < 0.001$ ) increase attributable to the addition of streptomycin. In comparison to the actual difference, 8.7 N. R. per cent, the weighted mean difference was 9.7 N. R. per cent. Table 1 shows the raw percentages and differences for the individual bulls.

The response of individual ejaculates to the streptomycin treatment was found to have a definite inverse linear relationship to the level of fertility of untreated samples. This relationship, which is graphically presented in figure 1, can be expressed as  $Y = 51.872 - 0.8876X$ .

TABLE 1  
*Percentage of non-returns by bulls*

	No. of ejaculates	Without streptomycin	With streptomycin	Difference
Doug	6	67.9	77.6	9.7
Major	8	61.5	65.4	3.9
Judge	6	73.0	78.2	5.2
Norman	5	66.9	64.5	-2.4
Conqueror	2	54.7	59.3	4.6
Kenneth	9	51.7	68.9	17.2
Fayne	5	60.6	74.8	14.2
Prince	8	67.1	66.1	-1.0
Nutmeg	3	65.8	68.6	2.8
Donald	9	47.9	68.2	20.3
Watchman	4	54.7	65.1	10.4
Impressive	11	60.7	69.3	8.6
Commander	2	64.3	89.1	24.8
Avalon	4	63.4	80.4	17.0
Mac	1	36.7	65.2	28.5
Superior	1	44.4	78.3	33.9

#### DISCUSSION

Mixner (7) reported at the same meeting that a preliminary report of this work (5) was presented that the addition of 1,000  $\gamma$  of streptomycin plus 1,000  $\gamma$  of penicillin per milliliter of diluted semen failed to result in a statistically significant increase in fertility. Four possible explanations for the discrepancy between Mixner's report and the data presented here have occurred to the authors. First, the calcium-chloride complex of streptomycin was used in his study, the sulfate salt in this one. Second, the 1,000  $\gamma$  level treatment with streptomycin may not be as favorable as a lower level. Third, streptomycin alone may be more effective in increasing fertility rates than a combination of streptomycin and penicillin. Fourth, in light of the association between initial fertility and response reported herein there is the possibility that the ejaculates in Mixner's report were of higher initial fertility and would not show as great a response under such circumstances.

The third possibility is supported by a comparative study conducted by Almquist (2, 3) in which semen from some relatively infertile bulls was treated with

streptomycin, penicillin and a combination of both. All treatments produced highly significant fertility increases as compared to controls. Although no statistically significant difference was found between the antibiotics used, the results

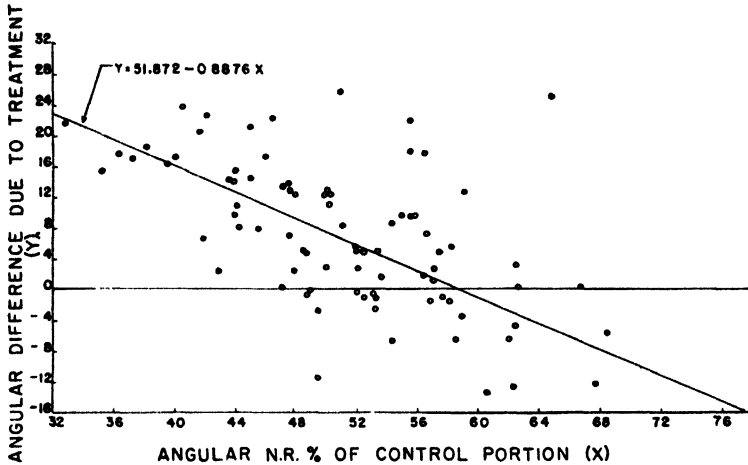


FIG. 1. Linear regression associated with streptomycin treatment of diluted semen.

did show that the percentage of conceptions for the streptomycin-treated semen was somewhat higher than semen treated with penicillin or the two in combination.

#### SUMMARY

Streptomycin sulfate in the amount of 100  $\gamma$  per milliliter was added to diluted bull semen used in the routine operation of an artificial breeding establishment on a split sample control basis for a period of 4 mo. A total of 2,340 cows was inseminated with the control semen, and 2,379 cows with semen containing streptomycin.

Over-all results showed a 61.1 N. R. per cent for the control group as compared to 69.8 N. R. per cent for the streptomycin-treated group. The increase of 8.7 N. R. per cent was found to be highly significant.

An inverse linear relationship between response and the initial level of fertility was noted even though most bulls of "so-called" high efficiency responded in some degree to treatment.

#### ACKNOWLEDGMENT

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# THE EFFECTIVENESS OF SOME ANTIFOAMING AGENTS IN THE CONDENSING OF SKIMMILK AND WHEY

J. ROBERT BRUNNER

*Michigan Agriculture Experiment Station,<sup>1</sup> East Lansing*

The foaming of skimmilk is a problem encountered in many dairy plant operations. The inherent tendency for skimmilk to foam results in the loss of both product and labor. One of the more obscure problems encountered in processing skimmilk and whey is the formation of large quantities of a very stable foam in the vacuum-condensing operation. The so-called "wild pan," a condition most prevalent in the spring of the year, is characterized by excessive foaming in the vacuum pan accompanied by a "boiling over" into the condensing unit. Ultimately, these milk solids enter the sewage system where their presence constitutes an additional problem of disposal. Although the "wild pan" is not a common experience in the larger commercial operations, the frequency with which this condition is encountered by the small, batch-size pan operator warrants attention.

The phenomenon of foam formation in fluid dairy products has been an object of scientific investigations for many years. Leete (3) and Sanmann and Ruche (9) observed that milk and skimmilk possessed minimum foaming characteristics at temperatures ranging between 20 and 30° C. They both noted an increase in foam volume and stability at temperatures above 30° C. Rahn and Sharp (6), Ansbacher *et al.* (1), and more recently El Rafey and Richardson (2), as well as numerous other investigators have noted the destabilizing effect of small amounts of milk fat on the foam structure of milk, separated milk and whey. In fact, the practice of adding small amounts of cream or butter to a "wild pan" as a means of breaking the foam has been a common practice among pan operators for many years. Such procedures, however, have not always been entirely satisfactory in alleviating excessive foaming in the vacuum pan.

In other industries, chemical antifoaming agents have been employed to reduce foaming. Ross (7) listed some of the more widely used chemical antifoamants into groups according to their chemical types: alcohols, fatty acids, and fatty acid esters, combinations of amides and fatty acids, ethers, organic phosphates and silicones. These compounds are surface-active materials possessing a positive spreading coefficient and act specifically as anti-foamants when they are insoluble in the continuous phase of the system involved. The object of this paper is to report the results of a study of various antifoaming agents added to skimmilk and whey.

## EXPERIMENTAL

A laboratory-model vacuum pan was assembled and is illustrated in figure 1. On controlled operation trials, which consisted of condensing 2 lb. of skimmilk to 27 per cent total solids, performance data scaled to production size pans were obtained. Skimmilk, separated at 95° F., or whey, obtained from a starter and

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rennet set whole milk, was heated in the hot well (*B*) to 160° F. and then admitted slowly to the vacuum flask under a vacuum varying between 25 and 26 in. of Hg. The boiling temperature for normal operating conditions was maintained at approximately 125° F. Under these conditions, 2 lb. of fluid skimmilk could be condensed in 2 hr. The profuse foaming of skimmilk and whey, comparable to that observed in the operation of production units, was induced by increasing the rate of intake into the vacuum flask until the vacuum was lowered from 26

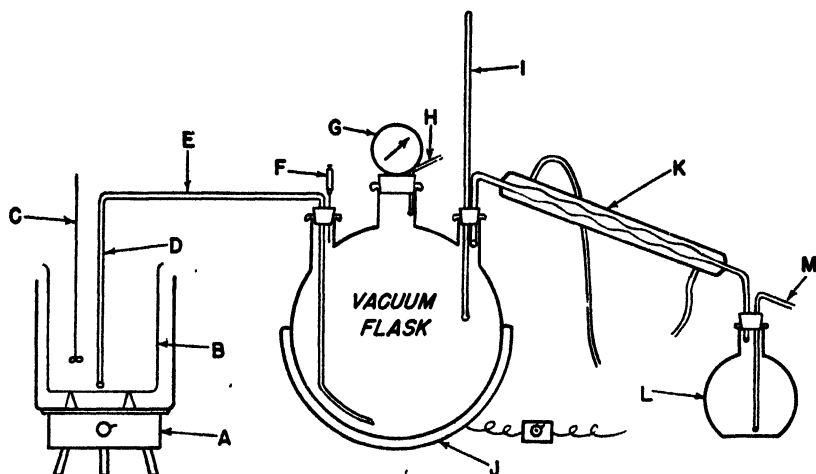


FIG. 1. Diagram of the laboratory-model vacuum pan used in this study.

- |                           |                                |
|---------------------------|--------------------------------|
| A. Hot plate              | H. Vacuum release valve        |
| B. Hot well               | I. Thermometer                 |
| C. Mechanical agitator    | J. Glas-Col heating mantle and |
| D. Intake to vacuum flask | rheostat control               |
| E. Intake control valve   | K. Condenser                   |
| F. Syringe                | L. Water trap                  |
| G. Vacuum gauge           | M. To vacuum pump              |

to 24 in. At this point, the intake was reduced to its normal rate. The rather sudden introduction of a relatively large volume of fluid containing a considerable quantity of dissolved air resulted in the production of copious amounts of a very stable foam, characteristic of foaming conditions observed in "wild pans."

The materials used as antifoaming agents were added directly to the vacuum flask by means of a medical syringe (*F*) protruding through a rubber stopper into the vacuum flask and are listed categorically in table 1 as (*A*) naturally occurring foam depressants and (*B*) chemical antifoaming agents. The naturally occurring materials represent antifoamants obtainable from natural sources which have been used to a limited extent as a means of alleviating excessive foam formation in the vacuum pan. The materials designated as chemical antifoaming agents were selected surface active materials representative of a large group of commercially available antifoaming agents frequently used in industrial operations as a means of combating undesirable foaming.

TABLE 1

*The apparent effectiveness of selected antifoaming materials when added to skimmilk and whey in the vacuum-condensing operation*

Materials studied	No. of trials	Concentration <sup>a</sup>	Antifoaming action <sup>b</sup>	Remarks
<b>A. Naturally occurring foam depressants</b>		(%)		
1. Milk fat				
a. As butter	2	0.5	+	The slight antifoaming action of butterfat is forfeited when the concentration exceeds 10%. The high fat concentration is conducive to the production of a stable frothy type foam.
	2	2.0	++	
	2	10.0	0	
b. As fresh cream	3	0.5	+	The high fat concentration is conducive to the production of a stable frothy type foam.
	2	2.0	+	
	2	10.0	0	
c. As rancid milk and/or cream	3	0.05	++	Very effective antifoaming action was noted. A slightly bitter flavor was detected in the highest concentration.
	2	0.1	+++	
	2	2.0	+++	
<b>B. Chemical antifoaming agents</b>				
1. Water insoluble materials				
a. Silicone compound	2	0.01	+++	Very effective and persistent antifoaming action. Off-flavor slightly perceptible in highest concentration.
	2	0.05	+++	
	2	0.10	+++	
b. Mono and diglyceride mixtures (glyceryl esters of stearic acid)	2	0.05	++	Dispersed in skimmilk at 160° F. Very effective antifoaming action except in lowest concentration. No off-flavor.
	2	0.1	++	
	2	0.2	+++	
c. Sorbitan monolaurate	2	0.1	+	Antifoaming action not persistent in lower concentrations. No off-flavor detected.
	2	0.3	++	
	2	0.5	+++	
	2	1.0	+++	
d. Octyl alcohol (2-ethyl hexanol)	2	0.01	++	Very effective antifoaming action was noted. Antifoaming tendency was not persistent. Sweet flavor detected in finished product.
	2	0.1	+++	
2. Water soluble materials				
a. Butyric acid	3	0.05	++	Antifoaming action accompanied by a general precipitation of the foam-film.
	2	0.1	+++	
b. Quaternary ammonium chloride (cationic)	2	0.05	0	Antifoaming action accompanied by a partial precipitation of the foam-film.
	2	0.1	++	
c. Alkyl-aryl sulfonate (anionic)	3	0.1	0	Foam formation is enhanced.
	2	0.5	—	
d. Polyoxyethylene sorbitan monolaurate (nonionic)	2	0.1	0	Foam formation is enhanced.
		0.5	—	

<sup>a</sup> Concentrations of antifoaming materials were based upon the initial weight of fluid skimmilk or whey.

<sup>b</sup> Antifoaming action was judged by observation and assigned relative values according to the following legend:

— increase in the foaming tendency  
 0 no apparent antifoaming action  
 + slight antifoaming action  
 ++ moderate antifoaming action  
 +++ pronounced antifoaming action  
 ++++ very effective antifoaming action



In addition to the effectiveness of these materials as antifoaming agents in the vacuum-condensing of skimmilk and whey, their use also was noted relative to the physical appearance and flavor of the finished product. For this purpose 0.5-pt. samples of the condensed product were stored at 45° F. for 24 hr. prior to judging for flavor defects. Flavor criticisms were made by two judges working independently.

#### RESULTS

Data and pertinent observations relative to the use of both naturally occurring foam depressants and chemical antifoaming agents in the alleviation of excessive foaming during the vacuum-condensing of skimmilk and whey are presented in table 1.

#### DISCUSSION

Surface activity in itself apparently is not sufficient evidence for assigning antifoaming properties to chemical substances, although Ross (7) stated that nearly every type of surface active material will act as an antifoamant if appropriately used. Whether or not the substance is soluble in the water phase is, obviously, of more significance in evaluating the antifoaming characteristics of a compound than is the relative surface activity as expressed in dynes per cm<sup>2</sup>. Parkhurst (5), studying the foaming characteristics of various surface active materials, advanced the thought that foam stability is not a function of low surface tension, as such, but rather of the adsorbed layer giving rise to the lowering of surface tension. Any postulation advanced to explain adequately the specific behaviors of surface active antifoaming agents must, therefore, include not only a consideration of surface activity but also the solubility characteristics, the nature of ionization and the electrostatic charge and other constitutive properties of the compound.

The ineffectiveness of water-soluble, anionic and nonionic surface active materials as antifoamants conceivably might be accounted for if it is assumed that the surface-active anions or nonionic molecules are oriented at the air/liquid interface (foam-film) in a definite scheme compatible with the surface active milk proteins. In fact, the addition of materials of this nature enhance the formation of a stable film and make possible the production of a foam showing greater volume and stability. If the stability of the foam structure is dependent upon the orientation of surface-active materials at the air/liquid interface, an assumption generally supported by existing evidence concerning the nature of foam formation, then any surface-active substance possessing characteristics opposed to those of the materials making up the foam membrane will disrupt the orientation pattern and cause the foam structure to disintegrate. Evidently, this was the result when a surface-active, cationic quaternary ammonium compound was added to the skimmilk. This compound is water-soluble and completely ionized with the surface-active ion bearing a positive charge. When this positively charged ion is oriented into the foam-film at the air/liquid interface in proximity with the negatively charged, surface-active milk protein, there exists a tendency for the mutual attraction of these ions which results in the partial precipitation of the protein

material and finally a collapse in the foam structure. The complete degeneration of the foam structure and the partial precipitation of the milk proteins upon the addition of small concentrations of butyric acid to the vacuum flask is explained in a similar manner. The positively charged hydrogen ions disassociated from butyric acid discharge the negatively charged protein sol causing it to precipitate into a gummy mass which inhibits the formation of foam. These characteristic behaviors of water-soluble surface-active materials when added to skimmilk or whey in the vacuum flask preclude the use of such materials as antifoaming agents.

The fact that all of the effective antifoaming materials studied in this work were water-insoluble, surface-active substances suggests the probability that these compounds are antagonistic to the systematic orientation of the milk proteins, presumably necessary for the formation of an elastic film, and consequently, a stable foam structure. Being water insoluble, molecular aggregates of these substances tend to displace the less surface-active milk proteins at the air/liquid interface. Since there is no tendency on the part of molecules of these antifoaming agents to arrange themselves into a definite pattern and an elastic film, the foam structure is broken.

The foam depressing effectiveness of milk fat in fresh cream or butter usually is attributed to the ability of free or globular fat, when carried into the foam lamella, to establish weak points which cause a collapse of the foam structure. This reasoning, however, cannot account for the increased foaming tendency of high fat creams. Neither can the decidedly increased antifoaming activity of much smaller amounts of rancid milk and cream be accounted for in this manner, but rather, on the basis of the substances produced as a result of enzymatic hydrolysis. Probably the most significant substances produced by enzymatic hydrolysis of triglycerides from the standpoint of antifoaming activity are the mono and diglyceride mixtures and free fatty acids. In view of the results obtained in this study with butyric acid, it is assumed that the presence of the water soluble, short-chain, fatty acids cannot account for the antifoaming activity of rancid milk fat. If butyric or other of these low molecular weight acids were liberated in any appreciable quantity, their disassociation would build up the hydrogen ion concentration to the point where a general precipitation of the foam-film would occur. The fact that such a precipitation did not result from the addition of small portions of rancid milk and cream enables us to discount the antifoaming action attributed to this class of fatty acids. On the other hand, the presence of the longer chain, water-insoluble acids as well as the residual mono and diglyceride mixtures, compounds possessing known antifoaming properties, probably explains the antifoaming effectiveness of rancid milk and cream.

All of the chemical antifoamants used successfully in this study, with the exception of the silicone, consisted of components found in combined or uncombined states in many common edible foods. Silicone compounds are non-toxic, however, when used in concentrations recommended by Rowe *et al.* (8). At the present time, many of the fatty acid esters find a popular use as emulsifiers in ice cream but they could serve a double purpose if they were added to skimmilk,

prior to condensing for serum-solids, to control excessive foaming in the vacuum pan.

#### SUMMARY

A laboratory model vacuum pan, designed to give operational performance comparable with production size units, was employed to study the effectiveness of various materials as antifoaming agents in the condensing of skimmilk and whey.

Milk, cream and butter as well as selected surface-active, chemical compounds were added to skimmilk and whey during the condensing operation. Cream and/or butter were moderately effective as foam depressants. Slightly rancid milk and/or cream were extremely effective foam inhibitors. Of the chemical antifoaming agents studied, the silicone compound, mono and diglycerol esters of stearic acid and sorbitan monolaurate constitute those used most effectively to eliminate the excessive foaming tendency of skimmilk and whey. These materials are water insoluble, surface-active substances.

#### ACKNOWLEDGMENT

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## THE EFFECTS OF FEEDING PARATHION TO DAIRY COWS<sup>1, 2</sup>

PAUL A. DAHM, F. C. FOUNTAINE, J. E. PANKASKIE,  
ROGER C. SMITH AND F. W. ATKESON

*Kansas Agricultural Experiment Station, Manhattan*

The increasing use during the past few years of synthetic organic chemicals for the control of insects on forage crops has led to considerable concern about the persistency of residues of these materials and the possible excretion of these chemicals in the milk. The appearance of DDT and related chlorinated hydrocarbon insecticides in the milk of cows whose diets included small amounts of these materials has been established (2, 4, 5, 6, 7, 12, 13, 14, 15, 17, 18). This contamination of milk has been the basis for certain precautions with regard to the use of DDT and related insecticides on forage crops that might be fed to lactating cows.

The organic phosphate insecticide known as parathion, 0,0-diethyl 0-p-nitrophenyl thiophosphate, possesses several unusual chemical and insecticidal properties. This material originated in Germany and subsequently has been produced commercially in this country. Parathion has proved to be effective in controlling a large number of insects and related arthropods. It has been demonstrated that amounts of parathion necessary for satisfactory insect control on such crops as peas, corn and alfalfa usually result in parathion residues of less than one part per million when analyzed 1 wk. or more after the last parathion application (1, 8, 9, 10, 11). Its insecticidal value is marred only by its high toxicity to warm-blooded animals. Because of the widespread use of parathion as an insecticide on crops which subsequently are fed to dairy animals, it was considered important to determine whether parathion included in the diet in amounts comparable to that which has been found as a residue on foliage would appear in the milk of dairy cows.

### PROCEDURE

Ten cows, including two Holsteins, four Guernseys, two Jerseys and two Ayrshires in mid-lactation were allotted to two equal groups on the basis of breed, stage of lactation and milk production. All cows were on good sudan, brome or rye pasture during the experiment. Atlas sorgo silage was fed at a level of 2 lb. per 100 lb. body weight, and the cows had access to alfalfa hay during the time of day when they were not on pasture. A grain concentrate mixture containing approximately 16 per cent crude protein was fed according to milk production. Cows in group I were fed commercially available parathion in the form of a 25 per cent wettable powder at the level of five parts per million of the estimated dry matter intake of the roughage in the ration. Cows

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in group II were fed parathion at the level of one part per million of the estimated roughage dry matter intake. The parathion was administered daily in capsules. Since forage crops treated with amounts of parathion for good insect control usually contain residues of less than one part per million, the feeding levels used in this study represent an intake of parathion greater than that which normally would be ingested with parathion-treated roughage. The maximum roughage dry matter intake of the cows was estimated to be 2.25 lb. per 100 lb. of body weight. On this basis, cows in group I were fed 0.112 mg. of parathion per kilogram of body weight, and cows in group II were fed 0.022 mg. of parathion per kilogram of body weight. All cows were weighed on 3 consecutive days at bi-weekly intervals. The average of these weights was used as the basis for calculating the parathion dosage for the ensuing 2 wk.

Ten of the cows were fed parathion continuously for 81 days. In order to study the effects of parathion feeding on cows producing small amounts of milk, two Ayrshires in the terminal 2 wk. of lactation were allotted to the two groups. These two cows in late lactation were fed parathion for only 14 days. At the termination of the 81-day period, the feeding level of two cows from group I, a Guernsey and an Ayrshire, was increased immediately to 10 parts per million of parathion on the same basis. Then, the amount of parathion fed was doubled each succeeding week until the cows were ingesting 40 parts per million daily. At this final level, which was fed for 1 wk., the cows were receiving approximately 0.9 mg. of parathion per kilogram of body weight daily.

Samples of carefully mixed milk were taken on alternate days for 6 days before the beginning of the parathion-feeding experiment and on alternate days for 6 days following the first feeding of parathion; after this, samples were taken twice weekly for 3 wk. Thereafter, samples were taken once a week for the duration of the experiment. The milk from the Guernsey and Ayrshire cows from group I, which were fed the higher levels of parathion, was sampled and analyzed twice weekly during the 3-week experiment.

Samples of milk from each cow were taken at frequent intervals and examined organoleptically to determine whether the feeding of parathion as a supplement to an otherwise normal ration would result in any detectable off-flavor in the milk.

#### ANALYTICAL PROCEDURE

Application of the sensitive colorimetric method of Averell and Norris (3) for estimating small amounts of parathion was tried on 100-g. samples of milk to which known amounts of parathion were added; extraction attempts were made using the methods developed by Schechter *et al.* (13) and Carter (5). The presence of interfering substances and a very low recovery of the parathion added to the milk did not permit the use of either of these established extraction methods. Upon the suggestion of Averell and Norris of the American Cyanamid Co., a new procedure was tried involving the use of a liquid-liquid extraction apparatus (fig. 1). Essentially, this procedure involves a prolonged percolation of petroleum ether through a column of milk and ethyl alcohol. The mixture in the extraction chamber was stirred at 30-min. intervals with a

wire-loop stirrer inserted through the Liebig reflux condenser. The liquid-liquid extraction procedure was carried out for an optimum period of 6 hr.

Details of the modified analytical procedure are as follows: A mixture of 100 g. of milk and 100 ml. of 95 per cent ethyl alcohol was placed in the liquid-liquid extraction apparatus. A pinch of NaCl, reagent grade (approximately 0.5 g.) was added to the milk mixture to prevent emulsion formation in the extraction chamber and the overflowing of milk solids into the Erlenmeyer

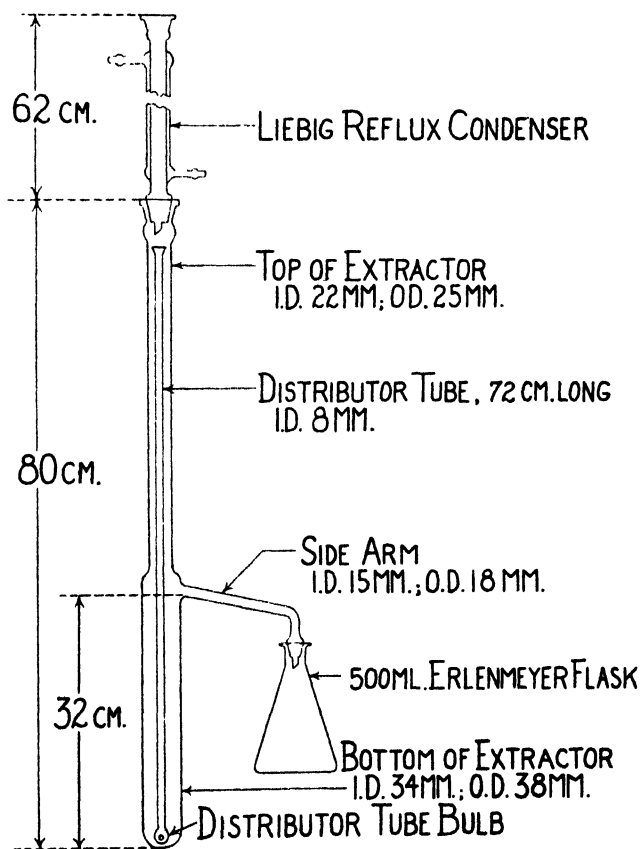


FIG. 1. Extraction apparatus for the determination of parathion in milk.

flask; 150 ml. of technical petroleum ether (Skellysolve "B") were placed in the 500 ml. Erlenmeyer flask of the extraction apparatus. After the assembled extraction apparatus was mounted, the petroleum ether in the Erlenmeyer flask was heated to gentle boiling with a 175-watt hot plate. The extraction process was carried on for 6 hr.; this was found to be the optimum time for best recovery of parathion. The solution in the extraction chamber was stirred at 30-min. intervals with the wire-loop stirrer.

After the extraction was completed, the petroleum ether was transferred to a 150 ml. beaker and evaporated almost to dryness on a warm water bath; a gentle stream of air passing over the liquid facilitated this process.

After evaporation, 20 ml. of 95 per cent ethyl alcohol, 20 ml. of water, 2 ml. of 5 *N* HCl and 0.2 g. of zinc dust were added. The beaker was covered with a watch glass and heated to gentle boiling for 10 min. The beaker was allowed to cool, then the watch glass was washed with distilled water; 25 ml. of petroleum ether were added to the beaker and the solutions swirled around gently to

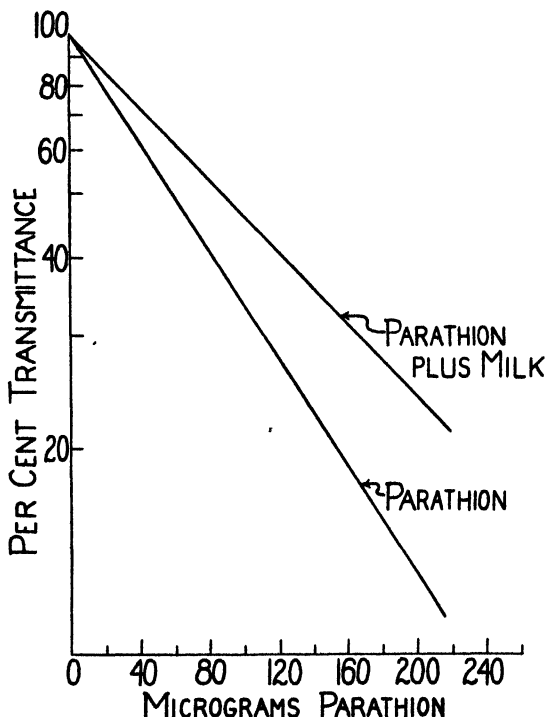


FIG. 2. Parathion standardization curves (Averell and Norris Method, 1948); calibration curves at 555  $m\mu$  using a Coleman Model 14 Spectrophotometer.

extract the fat. The ether layer was siphoned off and the extraction was repeated twice more, using 25 ml. of petroleum ether each time. After the third extraction, the last traces of petroleum ether were evaporated by means of a gentle stream of air. The solution then was filtered through no. 42 Whatman filter paper into a 50-ml. volumetric flask. The subsequent steps in the procedure were exactly the same as those reported by Averell and Norris (3).

Standard curves were prepared from the data obtained by adding known amounts of parathion (98.2 per cent parathion), ranging from 20 to 200  $\gamma$ , to 100-g. samples of milk. The per cent transmittance values were obtained using a wave length setting of 555  $m\mu$  with a Coleman model 14 spectro-

tometer. Standard curves for parathion and milk plus parathion, obtained by using this procedure, are shown in fig. 2.

Technical petroleum ether (Skellysolve "B") was used in all the analyses, since it was found that the use of petroleum ether purified according to the method reported by Werner (16) did not improve the results. Use of 95 per cent ethyl alcohol and distilled water to dissolve the residue remaining after evaporation of the petroleum ether prevented the formation of a turbidity that developed when benzene was used. Any slight turbidity appearing upon the addition of water to the solution of the residue dissolved in ethyl alcohol either disappeared during the reduction process or was removed by filtration.

Milk blanks varied from 95 to above 100 per cent transmittance when compared with the reagent blanks; therefore, in the actual analyses no reading above 90 per cent was considered significant. It can be seen from the standardization curves for parathion plus milk that a transmittance reading of 90 per cent using a 100-g. sample of milk would represent less than 0.2 of a part per million of parathion.

#### RESULTS

Approximately 250 separate analyses for parathion in the milk from parathion-fed cows were made during the course of the experiment. At no time was any parathion found in the milk from any of the cows fed parathion. These results were checked further by biological assays of the milk from the parathion-fed cows using adult houseflies (*Musca domestica*). The absence of any mortality among the flies served to confirm the negative analytical findings. Referee samples of milk from the parathion-fed cows were frozen and sent by air express to the Stamford, Conn., laboratories of the American Cyanamid Co. for analysis, where essentially the same analytical procedures were used. The results obtained in that laboratory also indicated that no parathion was found in the milk from cows fed parathion at a level of 5 ppm. of the dry roughage intake. A detailed description, treatment and response, of each of the cows fed parathion is shown in tables 1 and 2.

The organoleptic examinations of milk from the parathion-fed cows did not reveal any flavor defects that might be caused by the parathion.

The amounts of parathion administered to the experimental cows apparently had no deleterious effect on their health. Their appetites remained good and their general condition was such that there was no indication of toxic effects of the chemical. Cow no. 458A, a Guernsey that received the high levels of parathion in the final week of the experiment, became lame in her shoulder during the week in which she received parathion at the rate of 40 parts per million of the estimated roughage dry matter intake. In the opinion of the attending veterinarian the lameness was caused by a bruise. Since the cow's appetite remained normal, and her eyes bright, it was felt that the lameness probably was not caused by parathion. The absence of symptoms in the other cows would seem to support the contention that parathion did not cause the lameness.



TABLE 1  
Description, treatment and response of cows fed parathion for 81 consecutive days.

Group and no. of cow	Breed	Av. daily milk prod.	Days in lactation at beginning of expt.	Av. weight	Average daily estimated roughage dry matter intake	Av. daily parathion intake	Parathion intake as ppm. of roughage consumed	Av. daily parathion intake per kg. of body weight	Ratio parathion intake to kg. milk produced	Parathion recovered in milk
		(lb.)		(lb.)	(lb.)	(mg.)	(ppm.)	(mg.)	(mg./kg.)	(ppm.)
<b>Group I</b>										
122A	Holstein	37.7	177	1429	32.1	72.8	5.0	0.112	4.25	0
458A	Guernsey	25.8	152	1037	23.3	52.9	5.0	0.112	4.52	0
451A	Guernsey	26.6	160	1023	23.0	52.2	5.0	0.112	4.32	0
378A	Jersey	24.8	111	896	20.2	45.7	5.0	0.112	4.06	0
200A	Ayrshire	14.4	130	971	21.8	49.4	5.0	0.112	7.54	0
271A*	Ayrshire	16.0	234	1026	23.1	53.0	5.0	0.113	7.28	0
<b>Group II</b>										
133A	Holstein	45.5	161	1332	29.9	13.3	1.0	0.022	0.64	0
469A	Guernsey	28.2	131	1100	24.8	11.0	1.0	0.022	0.86	0
465A	Guernsey	27.3	153	1025	23.1	10.2	1.0	0.022	0.82	0
358A	Jersey	22.3	95	846	19.0	8.4	1.0	0.022	0.83	0
246A	Ayrshire	29.7	121	1150	25.9	11.5	1.0	0.022	0.85	0
268A*	Ayrshire	10.7	364	1137	25.6	11.0	1.0	0.021	0.95	0

\* Parathion fed only during the terminal 2 wk. of lactation.

TABLE 2  
Description, treatment and response of two cows fed parathion daily in amounts increased at weekly intervals from 5 to 40 ppm.

No. of cow	Breed	Week of feeding	Average daily milk prod.	Average daily weight	Average daily roughage dry matter intake	Average daily parathion intake	Parathion intake as ppm. of roughage consumed	Average daily parathion intake per kg. of body weight	Ratio parathion intake to kg. milk produced	Parathion recovered in milk
			(lb.)	(lb.)	(lb.)	(mg.)	(ppm.)	(mg.)	(mg./kg.)	(ppm.)
458A	Guernsey	1	20.7	1061	23.9	54.1	5.0	0.112	5.8	0
		2	20.2	1060	23.9	108.2	10.0	0.225	11.8	0
		3	19.0	1068	24.0	216.2	20.0	0.446	25.1	0
		4	17.2	1068	24.0	432.4	40.0	0.892	55.4	0
200A	Ayrshire	1	10.8	983	23.1	50.1	5.0	0.112	10.2	0
		2	9.5	972	21.9	100.2	10.0	0.227	23.2	0
		3	8.9	975	21.9	198.3	20.0	0.452	49.1	0
		4	7.9	975	21.9	396.6	40.0	0.897	110.8	0

All of the cows gained slightly in body weight during the course of the experiment (fig. 3); such weight gains are to be expected in cows in that stage of lactation. It is apparent, therefore, that parathion in the amounts fed had no apparent effect on body weight.

Average milk production of the cows in both groups is shown also in fig. 3. Cows in group I had been in lactation an average of 146 days and those in group II, 132 days, at the beginning of the experiment. The slope of the lactation curves is normal for cows in the latter stage of lactation. At the levels fed it would seem that parathion had no deleterious effect on milk production

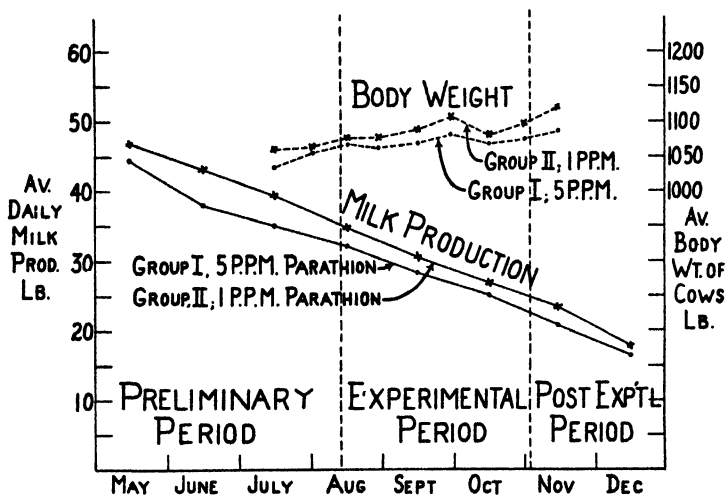


FIG. 3. Average monthly milk production and body weights of cows.

Of the 12 cows used in the experiment, eight were pregnant before parathion was fed and three were bred and conceived shortly after the trial started. The remaining cow came into heat regularly, but was not bred. All pregnancies have been normal and all calves born have been strong and vigorous at birth.

#### DISCUSSION

Parathion is being used successfully as an insecticide on forage crops at the rate of 0.5 lb. or less of actual parathion to the acre. Under these conditions of use, normally several days will elapse between the application of parathion for insect control and harvesting the crop. A review of published data pertaining to parathion residues either in or on crops such as alfalfa, beans, inner leaves of cabbage, corn leaves, corn stalks, whole peas and pea vines reveals no case where parathion was found to exceed one ppm. of the plant involved when the analyses were carried out from 7 to 21 days after the last application of parathion (1, 8, 9, 10, 11). Furthermore, directions for using parathion explicitly caution against applying the insecticide to crops later than 30 days before harvest.

At the rate of 22.5 lb. of dry matter ingested daily per 1000 lb. of body weight, a dairy cow would consume the equivalent of 90 lb. of fresh green alfalfa. Such a quantity, with a residual parathion rate of one ppm., would cause the intake of parathion to be approximately 40 mg. daily. This does not take into consideration the highly volatile nature of parathion which has been established by residue analyses following the application of parathion to forage crops (8, 9, 11). Therefore, it might be expected that much less than the approximately 40 mg. of parathion present in the freshly cut alfalfa would be present when the hay was consumed. The one ppm. parathion residue on alfalfa therefore is less than the amount ingested by the experimental cows which were fed at a level of 5 ppm. of the estimated dry matter consumed. Also, two of the cows were fed more than eight times that amount for 1 wk.

The fact that the health of the cows seemed unimpaired at any of the dosages used or any cumulative ill effects resulted from rather extensive feeding periods seems to indicate rather conclusively that the crops sprayed with parathion at the usual rates for insecticidal purposes are not made unsafe for livestock feed.

The possibility of deleterious amounts of parathion in the milk produced by such fed animals is a more critical question. By the analytical methods used no parathion was recovered in any of the milk from the cows fed at different known levels, some of which were excessive. The efficacy of the analytical method might be questioned as a precautionary measure by health officials. However, the fact that the milk was tested biologically by feeding it to houseflies with no measurable effect seems to clinch the fact that the parathion was not coming through into the milk.

Two other facts seem worthy of mention. First, the experimental periods involved gave no evidence of cumulative effects on the health of the animals or their milk production. Second, the ratio of parathion fed to milk secreted varied from 0.64 mg. of parathion per kilogram of milk to nearly 12 times that amount in the lower-producing animals. These facts further indicate the reasonable safety of using milk from such parathion-fed dairy cows.

Since the parathion intake of the animals was controlled for extensive periods at what seemed to be higher than expected levels and since no parathion was recovered in the milk at any time, one might raise the question as to the fate of the parathion in the animal body. The authors obtained no data to answer this question. Research pointed toward answering this question not only would be of scientific interest but might establish further the relative safety of using feed containing parathion, provided it could be established that the parathion, or much of it, could be accounted for in other secretions of the body.

#### SUMMARY

An experiment was designed to determine the presence or absence of parathion in the milk of dairy cows fed parathion in capsules. Ten dairy cows in heavy lactation, representing four of the major breeds, were allotted into two groups and fed a commercially available wettable powder formulation of para-

thion at levels of five ppm. and one ppm., based upon an estimated roughage dry matter intake of 2.25 lb. per 100 lb. of body weight daily, continuously for 81 days. These feeding levels represent an actual parathion intake of 0.112 mg. of parathion per kilogram of body weight for the cows receiving five ppm. of parathion, and 0.022 mg. of parathion per kilogram of body weight for the cows receiving one ppm. of parathion based upon the estimated roughage dry matter intake. At the conclusion of this experiment, two of these cows were fed increasing amounts of parathion up to 40 ppm. of the estimated roughage dry matter intake. In neither experiment was any parathion found in the milk of the experimental cows by the use of both a chemical method of analysis for parathion and biological assay using adult houseflies. No objectionable flavor was noted in the milk and no harmful effects to the health or reproductive ability of the cows were observed.

#### ACKNOWLEDGMENTS

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# MILK FEVER (PARTURIENT PARESIS) IN DAIRY COWS—A REVIEW

J. W. HIBBS

*Ohio Agricultural Experiment Station, Wooster*

Milk fever (parturient paresis) is an afebrile disease which typically is associated with parturition and beginning lactation. It is characterized by a sudden paralysis, gradual loss of consciousness and, if untreated, usually terminates in death. Few diseases of livestock have caused as much theoretical controversy and interest as has milk fever. Gradually, through the years, much has been learned about the nature of milk fever, and effective means of treatment have been devised, resulting in a reduction in mortality of from 60 to 70 per cent to less than 1 per cent. The basic physiological cause of milk fever has yet to be proven. The "parathyroid deficiency (hypocalcemia) theory" of Dryerre and Greig (54) seems to come the nearest of the many theories that have been advanced to accounting for the immediate cause, but many fundamental questions remain unanswered.

## EARLY HISTORY

According to Fish (62) it is safe to assume that no such disease as milk fever existed two or three centuries ago. Hutyra and Marek (125) state that the time when the disease became known corresponds with the period when it became customary to feed cows more generously with the object of increasing milk production.

As intensive feeding and selection for higher milk production increased, the number of milk fever cases also increased until by the middle of the 19th century numerous publications on the subject by veterinarians were appearing in the literature from nearly all civilized countries. Hutyra *et al.* (126) have pointed out that milk fever was first mentioned in the literature in Germany by Eberhardt in 1793. Price (Hutyra *et al.*, 126) refers to milk fever in 1806 in his book "The New Useful Farrier and Complete Cow Leech." Skellet (Hutyra *et al.*, 126), an English veterinarian, gave a good clinical description of the disease in 1807, and it was particularly discussed by Jorg in 1808. According to Hutyra *et al.* (126), milk fever first was mentioned in French literature as late as 1837 by Fabre in his "Veterinaire Compagnard."

The treatment of milk fever in the early days was a reflection of the popular medical treatments of the period. Price (1806) (Hutyra *et al.*, 126) recommended sweating by the use of hot packs and blanketing. In 1814, Clater (38) recommended bleeding (4 to 5 qt. 8 to 10 days before calving) as a preventive measure. The following drink was recommended as a preventive to be given at night before turning out to pasture: 1 oz. alum, 1 oz. nitre, 1 oz. cream of tartar, 4 tablespoonsful treacle; after mixing, add to this 1 qt. of boiling ale and beer mixed; 2 hr. after giving, the beast can be turned out.

Boericke (31) in quoting Blaine, "Veterinary Art," p. 296, says, "The treat-

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ment of milk fever in the early stages alone calls for bleeding and that liberally." He also advised "a plenty of most powerful medicines." On the other hand, he quotes Gamgee ("Dairy Stock" Edinburgh 1861, p. 72) as saying "Above all things avoid strong remedies and bleeding. Either method seems equally unsuccessful, for nearly all die that are taken, according to the testimony of allopathic veterinarian surgeons." He recommends Belladonna (ten drops) and Nux Vomica.

In 1879, Teller (245) indicated that veterinarians were beginning to question whether or not milk fever and mastitis were synonymous. He recommended stimulants, purges, pouring cold water on the head and chloral hydrate to quiet excitable cases.

Navin (166), in 1872, attributed milk fever to an inflammatory disease of the womb or the peritoneum covering it. "Prompt and copious bleeding is the sheet anchor in this disease," plus sedatives. He also recommended rubbing the cold legs with cayenne pepper and alcohol.

Williams (271) in 1884 attributed milk fever to congestion of the brain and apoplexy associated with heavy milk production and parturition. As treatment he recommended bleeding to prevent congestion of the brain, physics, stimulants, etc. A statement of Williams' aptly expresses the seriousness of the disease to dairymen prior to the classical discovery of the udder inflation treatment in 1897 by Jurgens J. Schmidt of Kolding, Denmark (209). Williams says, "I may state in conclusion that parturient apoplexy (milk fever) is a recurring disease and that it is not safe to allow a cow to calve after it has once been down; and if the owner consults his own interests he will milk it as long as possible and then prepare it for the butcher."

#### MILK FEVER THEORIES

As is the case with most diseases whose cause is mysterious, numerous theories have been advanced from time to time to explain the etiology of milk fever. Many of these theories, when viewed in retrospect, appear ridiculous in the light of our present knowledge of the disease. However, these theories attracted wide attention at the time of their publication, and through study and observation, the false hypotheses gradually were eliminated. This process led to the present day understanding of the nature of milk fever as set forth in Dryerre and Greig's theory of parathyroid deficiency (hypocalcemia) (54) published in March, 1925, and confirmed by experimental evidence by Little and Wright (147) in May, 1925.

At this point it would seem desirable to summarize briefly the numerous theories that have been advanced in explanation of the etiology of milk fever ("the disease of theories"). The names of these theories, their chief adherents and a brief statement of their bases are as follows:

(1) *General inflammation.* Clater (39), Delabere Blaine (28), Youatt (275), Harrison (100) and Hering (114). This early theory attributed milk fever to congestion and inflammation, especially of the udder and brain. Bleeding and cold packs were recommended as a cure.



(2) *Derangements of the nervous system.* Contamine (42), Friend (73), Robinson (200), Ralph (194), Kohne (135), Rychner (204), Stewart (237), Binz (27), Füsich (74), Röhl (203), Baumeister-Rueff (21) and Gunther and Felizet (95). The paralysis of milk fever was thought to be caused by some mysterious derangement of the nervous system.

(3) *General circulatory disturbances.* Bredo (35), Pomayer (181), Kreutzer (137) and Seitter (222). According to this theory, the symptoms were due to low blood pressure brought on by an alteration of certain vasomotor centers. Udder insufflation was thought to act by relieving the low blood pressure.

(4) *Cerebral anemia.* Franck (69), Glass (79), Haubner (110), Wermer (261), Prehr (189), Aronsohn (11), Baroni (18), Zoppini (277), Dommerhold (53), McConnell (151), Billings (26), Hess (115), Meier (156), Sonnenberg (234), Gratia and van den Eeckhout (84), Spiegel and Spiegel (235) and Zehl (276). Cerebral anemia supposedly was caused by the increased supply of blood to the udder. Air insufflation of the udder was thought to relieve the situation. Hutyra and Marek (125), however, were unable to produce milk fever symptoms by removing as much as 44 per cent of all the blood from a cow.

(5) *Cerebral congestion.* Violet (254), Barlow (17), William Williams (270), Sanson (206), Cox (44), Barron (19), Noquet (173), Campbell (36), Ayrault (15), Trasbot (249) and Denenbourg (51). The treatment advised by those who accepted this theory was profuse bleeding, pouring cold water on the head, etc. The symptoms were attributed to congestion of the brain with blood.

(6) *Apoplexy.* Thacker (246), Festal (60), Bragnard (32), Coenraets (40), Walley (257), Beart Simonds (223), Ward (259), Devleeshower (52), Whincop (263) and Gerrard (76). From this theory developed the name parturient apoplexy, often used in early days to designate milk fever. It was believed that the paralysis was caused by cerebral hemorrhage (apoplexy).

(7) *Thrombosis.* Layman (142), Cox (43) and Wild (269). Milk fever, according to this theory, was due to paralysis caused by a blood clot (thrombosis).

(8) *Fat embolism.* Penberthy (179) attributed the paralysis and coma to the formation of a fat embolism as the name of the theory infers.

(9) *Spinal traumatism.* Rogerson (202) attributed the paralytic symptoms to injuries of the spinal cord.

(10) *Aeremia.* Harms (99) attributed milk fever to the presence of too much air in the blood.

(11) *General infection.* Allemani (7), Harrison and Thomas (101), Potiez and Concur (182) and van der Velde (252). This theory attributed the paralysis to the absorption of toxins of bacterial origin. No specific site of infection was indicated as in the following two theories.

(12) *Bacterial infection of uterine origin.* Rainaud (193), Pavesse (178), Wannovius (258), Lafosse (139), Stockfleth (238), Lanzillotti-Buonsanti (140), Zundel (278), Lyman (150), Schmidt-Mülheim (210), Friedberger and Fröhner (71), Pugh (192), Nochard (172), Guillebeau (94), Hess (115), Trinchera (250), Cozette (45), Lignieres (143) and Hartenstein (103). This theory cites the incriminating cause as being staphylococcic and streptococcic infection in the

uterus resulting in intoxication of the central nervous system, especially the medulla oblongata. This condition was different from puerperal septicemia in that in milk fever only an absorption of the toxins occurred, resulting in paralysis of the vaso-motor centers followed by congestion of the abdomen, uterus and udder with blood.

(13) *Infection of mammary origin.* Thomassen (247), Knusel (136), Hebbelyneck (112), Parker (176), Delmer (49) and Schmidt (209). According to Schmidt, materials are present in the colostrum which are products of cellular disintegration due to toxins of unknown bacterial origin. This was the basis of the potassium iodide-injection treatment. Knusel believed the beneficial effects of oxygen inflation of the udder resulted from the destruction of anaerobic bacteria, which were responsible for the toxins which caused milk fever. Mastitis doubtless was troublesome in making a differential diagnosis when this theory was advanced.

(14) *Anaphylaxis.* Huttyra and Marek (125), and Van Goidsenhoven (253). The basis of this theory was the formation of antibodies during the preceding lactation due to the absorption of milk casein. The infrequent cases of milk fever at the first parturition were explained on the basis that sufficient milk is secreted to cause absorption of milk casein in high producers, as early as 2 mo. before parturition. This theory was not confirmed experimentally by injection of placental emulsion of milk or of colostrum. Alexandrescu and Cinca (3) could not produce milk fever symptoms in cows in which anaphylactic shock was induced with casein.

(15) *Mammary-neurasthenia.* Wooldridge (273) attributed milk fever to nervous prostration brought on by mammary congestion.

(16) *Avitaminosis.* Bayard (22) believed the milk fever syndrome was due to vitamin B<sub>1</sub> deficiency.

(17) *Anhydremia.* Shock resulting from anhydremia is the basis of the anhydremia theory of Harding (97, 98). The author admits that this does not explain the low blood calcium and phosphorus values. This theory was advanced in 1929 after the hypocalcemia theory. The work of Wilson and Hart (272) indicates that anhydremia is not a factor in milk fever because of the very slight differences in blood protein between normal and milk fever cows. The work of Huttyra and Marek (125) and Abderhalden (Huttyra *et al.*, 126) tended to disprove this theory, as it was shown that profuse bleeding did not produce milk fever symptoms. Law (141) reports that the size of the red blood cells is reduced in milk fever, due to anhydremia, excess salt and albumin.

(18) *Auto-intoxication.* Albrecht (2), Friend (72), Hodges (121), Fischer (61), D. Pugh (191), Allemani (8), Abadie (1), Schutt (213), Kaiser (132), Rainaud (193), Stohrer (240), Wieners (268), Nash (165), Thompson (248), Delmer (50), Menig (158) and Healy and Kastle (111). Auto-intoxication caused by toxins from the pregnant uterus, kidneys, udder epithelial lining, colostrum and disintegration products of milk proteins have all been advanced as causes of milk fever. Illnesses similar to milk fever, but which develop independently of parturition also were explained on this basis. Menig (158) claims

to have produced milk fever symptoms by tying off the renal arteries of cows. Healy and Kastle (111) attempted to show a similarity of milk fever to eclampsia in humans without success.

(19) *Defective oxidation in the tissues.* Fish (62, 63) cites the possibility of defective oxidation in the tissues, of unknown cause, as the basis of milk fever.

(20) *Excess oxytocic principle in blood after parturition.* This theory, advanced by Bell and Morris (23, 24) in 1934, was based on the finding that milk fever cows had more oxytocic principle in the blood than did control cows, but it was not confirmed experimentally.

(21) *Faulty protein metabolism.* In 1936, Scott (214) attributed milk fever to faulty protein metabolism resulting from the high protein content of cow's milk. No experimental evidence was presented in support of this theory.

(22) *Ovarian dysfunction.* Smith (230) believed milk fever is due to ovarian dysfunction; however, his experiments were uncontrolled and very fragmentary. This theory was reported in 1946. In 1947, Smith (231) reported further on this theory.

(23) *Hyperfunctioning of the anterior pituitary.* From 1937 to 1940, Seekles (215, 216, 217) published a series of papers in which he demonstrated the presence of ketogenic, glycogenolytic and hyperglycemic factors in the blood of milk fever cows; these factors resembled the corresponding anterior pituitary hormones. He also demonstrated antiketogenic and hypoglycemic factors comparable to antibodies of these pituitary hormones.

(24) *Disturbed cholesterol metabolism.* Moussu (164) (Ilutyra *et al.*, 126) attributed milk fever to a decrease of blood cholesterol and acetone bodies due to beginning lactation. Hayden (106), however, showed that blood cholesterol does not increase following udder inflation and during recovery from milk fever.

(25) *Hyperadrenalinemia.* Zimmerman (Ilutyra *et al.*, 126) was of the opinion that milk fever is caused by an excess of adrenalin in the blood at parturition due to an endocrine upset.

(26) *Magnesium narcosis.* Kloubok (134), Pribyl (190), Schulhof (212) and Seekles and Sjollem (218). The magnesium narcosis theory is based upon the similarity of symptoms between milk fever and those observed when  $MgSO_4$  is injected into cows. Blood magnesium has been reported to increase in milk fever by many other investigators, including Allcroft and Green (6), Barker (16), Godden and Duckworth (81), Seekles *et al.* (221), Sjollem and Seekles (227, 228) and Hibbs *et al.* (120).

(27) *Alkalosis.* Craige *et al.* (46, 47, 48) concede that milk fever is a hypocalcemia but contend that this is brought on by alkalosis resulting from acid excretion into the colostrum, plus an alkaline diet. They recommend injecting chlor-ethamine (ethylenediamine dihydrochloride) along with calcium gluconate to relieve the alkalosis.

(28) *Acidosis and auto-asphyxiation.* Pierson (183) attributed milk fever to an acidosis that results in asphyxiation (lack of oxygen in the tissues).

(29) *Hypoglycemia.* Widmark (263), Widmark and Carlens (264, 265), Auger (12, 13, 14), Maguire (158) and Edwards (57). This theory will be dis-

cussed in detail later. In the course of experiments designed to prove or disprove this theory much knowledge has been added to the nature of milk fever.

(30) *Parathyroid deficiency (Hypocalcemia)*. Dryerre and Greig (54, 55), Greig (89, 90, 91), Barker (16), Fish (63, 64, 65, 66, 67), Godden and Duckworth (81), Allcroft and Green (6), Little and Wright (147, 148), Little (144), Little and Mattick (146), Seekles and Sjollema (218), Seekles *et al.* (221), Sjollema (224, 225), Sjollema and Seekles (227), Wilson and Hart (272), Petersen *et al.* (180), Hibbs *et al.* (117, 119, 120), Smith *et al.* (232, 233), Niedermier *et al.* (169, 170) and Blosser and Smith (29). This theory also will be discussed at length as it is the basis for much of our present understanding of the nature of milk fever.

Several reviews of the nature of milk fever appear in the literature. Thomasen (247), Dryerre and Greig (54), Little and Wright (147), Hutyra and Marek (125), Greig (89), Sjollema (226), Udall (251), Giltner (77) and Isherwood (127) are a few. The symptoms and treatments are described adequately in nearly all textbooks on diseases of cattle.

#### *The Schmidt (Udder Inflation) Treatment*

The first epoch in milk fever history occurred in the year 1897. In that year, a veterinarian by the name of Jurgens J. Schmidt (209) of Kolding, Denmark, brought forth his theory that the cause of milk fever was a virus infection of the udder. This theory resulted ultimately in the udder inflation treatment and immediately reduced the mortality hazard of milk fever from 60 to 70 per cent to about 15 per cent.

Schmidt's theory was based on a mistaken observation. In subjecting the colostrum of affected cows to microscopic observation, he noticed what he believed to be evidence of cellular disintegration and concluded that some ferment or toxin within the udder was responsible for the decomposition of the epithelial lining of the udder. He believed this was caused by a virus infection. What Schmidt probably saw were normal constituents of colostrum, colostrum bodies, or corpuscles of Donne (mononeuclear cells filled with fat droplets), which he would have discovered had he controlled his observations and examined the colostrum of normal cows as well.

In order to destroy the infection, he selected KI (1 per cent solution) and injected it into the udders of cows with milk fever. This treatment was remarkably successful and, when adopted, reduced the mortality of the disease to 15 per cent.

Other veterinarians found that different percentages of KI could be used, as well as 1 per cent, and that different amounts of the solution were equally effective in curing milk fever. Water alone likewise was effective.

Anderson and Evers of Skanderborg are said to have been the first to practice udder inflation with air alone in 1901. They found that oxygen was no more effective than air. With this discovery the mortality was reduced to about 1 per cent. A thorough discussion of udder inflation appears in Hutyra and Marek (125). Zehl (276) is credited with the development of the double air catheter

used in air inflation of the udder. Thus, through an erroneous observation and a false deduction, an effective cure of milk fever was discovered. However, the basic cause of milk fever was yet unknown; in fact, as Greig (89) stated, "the very specificity of the cure rendered the nature of the malady all the more obscure; yet the problem was the more attractive by reason of its apparent simplicity."

The Schmidt treatment and its modifications introduced a new hazard, that of mastitis, unless practiced with extreme aseptic precautions. This is emphasized by Jensen (129).

Except for minor variations in the Schmidt treatment and improvements in the apparatus for udder inflation, very little was done experimentally which expanded the knowledge of the nature of milk fever until about 1925. About this time two new theories were advanced, both of which have been subjected to extensive scientific investigation, namely, the Hypoglycemia and the Parathyroid deficiency (Hypocalcemia) theories. Although the former theory since has been refuted, the experiments carried out in connection with these two theories are largely responsible for our present understanding of the nature of milk fever.

#### *The Hypoglycemia Theory*

Nochard (171) in 1885 first noticed sugar in the urine of milk fever cows. He did not distinguish between glucose and lactose.

According to Neefs (167), this concept of milk fever had its origin in 1923 when a Canadian veterinarian, whose name is not recorded, was impressed by the similarity of symptoms between insulin shock and those of milk fever. It is said that he effected a cure in a milk fever cow by the intravenous injection of glucose. This never was recorded in the literature.

Widmark and Carlens (264, 265, 266, 267) and Widmark (263) have published a series of papers in support of the hypoglycemia theory. They attributed the disease to glucose deficiency brought on by the intensity of mammary secretion. Widmark suggests that it is a reaction to advanced breeding. Maguire (153) reported the successful use of intravenous injections of glucose in milk fever. He explained the action of glucose in milk fever thus: Insulin is a hydrator (causes protein particles in blood to enlarge and precipitate in the cerebral capillaries). Glucose, a dehydrator, causes the opposite effect and alleviates the symptoms produced by excessive insulin production in milk fever. Later in the same year, Maguire (154) abandoned this concept and agreed with Anger (12) and McLeod (152) that milk fever was caused by hypoglycemia resulting from removal of glucose by the mammary gland. Edwards (57) cited hypoglycemia after parturition in dogs in support of the hypoglycemia theory of milk fever.

Support was given by Hoyois (123) and Bru (34) to the view that milk fever is dependent upon an abundant milk secretion; they reported that repeated milking after parturition favored the appearance of milk fever. Hoyois (123) stated that milk fever did not occur if the cow had an attack of mastitis immediately after parturition, as this stops milk secretion.

The experiments of Porcher (184, 185, 186) and Kaufmann and Magne (133) have shown that lactose in milk originates from the blood carbohydrates and that lactose is readily absorbed back into the blood stream and is excreted in the urine. This was offered in explanation of the lactosemia observed in milk fever. Porcher and Panisset (187) state that colostrum is only a milk modified by retention. Porcher and Tapernoux (188) showed that lactose injected into the udder of a dry goat was largely absorbed into the blood stream.

Maguire (154) argues that intensive feeding will not cause milk fever and cites the practice of English breeders of feeding a high carbohydrate ration ("steaming") before parturition. This practice, while it increases milk secretion, also builds up the carbohydrate reserves and is said to prevent milk fever. Accordingly, mammary inflation was supposed to effect a cure by raising the blood sugar, as the experiments showed.

After the hypoglycemia theory had been advanced by these authors, papers by Hayden and Sholl (104), Hayden (105, 106), Fish (62, 63), Little and Keith (145) and Moussu and Moussu (163) showed that hyperglycemia was found in milk fever, rather than hypoglycemia. In 1926, Greig (85) criticized the hypoglycemia theory on the basis that the experimental evidence showed a hyperglycemia rather than a hypoglycemia. Auger (13, 14) replied that the high blood sugar values are due to a mixture of glucose, which is available to the tissues, and lactose, which is reabsorbed from the mammary gland and which is not available to the tissues. Thus, although an increase occurred in total sugar, the actual available sugar (glucose) is deficient in milk fever.

In 1926, no method was available for differentiating between the two sugars in the blood. It was reasoned by Greig (89) that if glucose was in excess, some would be found in the urine. This he was able to demonstrate in four out of 14 samples of urine from milk-fever cows. Amadon (9) reported that there is not sufficient hypoglycemia during milk fever to cause coma. This matter was conclusively cleared up by Hayden (105) when he was able to show a relative hyperglycemia in the blood of milk-fever cows using the Folin-Svedburg (68) method for differentiating between glucose and lactose in the blood and urine. Hayden (105, 109) showed, in addition to hyperglycemia, that lactose occurs only occasionally before mammary inflation but that the increase in total sugar of the blood after mammary inflation is due to reabsorbed lactose which is unavailable to the tissues and is excreted promptly.

These experiments conclusively showed that milk fever is not due to hypoglycemia and indirectly gave support to the hypocalcemia theory of Dryerre and Greig (54), which was advanced at approximately the same time.

Petersen *et al.* (180) later were unable to demonstrate milk fever symptoms when the blood sugar of cows was lowered to 17 mg. per 100 ml. by the injection of insulin.

Schlotthauer (208) in connection with a review of milk fever theories, especially the hypoglycemia theory, presented evidence that a hyperglycemia, rather than a hypoglycemia, is present in milk fever.

### *The Parathyroid Deficiency (Hypocalcemia) Theory*

The parathyroid deficiency (hypocalcemia) theory without supporting experimental evidence, was first published early in 1925 by Dryerre and Greig (54). According to Greig (89), he and Dryerre became interested in the milk fever problem in 1924 and resolved to undertake the investigation of the problem. Their approach was that of submitting the whole problem to a process of reasoning, after first collecting all the available evidence bearing on the occurrence and clinical manifestations of the disease. The results of this process of reasoning were presented (54) in the form of a working hypothesis, without experimental evidence to support it. The following are the main points considered in formulating the parathyroid deficiency (hypocalcemia) theory, as described by Greig (89):

"Commencing with the fact that specific cure resulted, no matter whether antiseptic fluids, sterile water, oxygen or air were injected into the udder, it seemed obvious that the effect, whatever it might be, depended upon the mechanical distention of the mammae.

"We then premised that simple distention of the mammae must act either:

- 1) by eliciting some endocrine disturbance, and/or
- 2) by mechanically retarding or arresting milk secretion, and so preventing the loss in the milk of some substance vital to the organism.

"That the disease was in some way closely associated with milk secretion was suggested by the facts that:

- 1) Its appearance as a clinical entity was coincident with the commencement of the development of the modern heavy milking strains.
  - 2) It was much more prevalent in dairy breeds as distinct from beef breeds.
  - 3) It very commonly attacked those individuals which specially possessed deep milking qualities.
  - 4) The period of greatest susceptibility in the milking life of an individual cow corresponded to the period of greatest milk secretion. Primiparae were very rarely affected.
  - 5) The rapid emptying of the udder by hand might precipitate the onset, while the practice of repeatedly removing small quantities of milk, or, alternatively, of permitting the calf to suck the cow for the first few days after calving, was recognized as a valuable preventive procedure.
- "The milk cow has been bred to produce an enormous quantity of milk; indeed, her milk secretion may now be regarded as almost pathological.

"The colostrum of the cow is rich in calcium, and it was considered likely that the onset of a profuse lactation might occasion a rapid reduction in the concentration of the blood calcium. This idea seemed to be supported by our further observations that the spastic seizures which often characterize the early stages of milk fever were tetanic in character.

"We believed that the more mechanical withdrawal of calcium from the blood as a result of the onset of a profuse secretion of milk could not in itself be regarded as the cause of milk fever, because, if this were so, every heavy milking cow would be subject to the disease.

"For that reason we postulated that some other factor, therefore, must act as a predisposing cause, and we suggested that such might be found in parathyroid dysfunction.

"The following, then, were the essential points in our hypothesis:

- 1) The nature of milk fever may be understood as a parathyroid deficiency, resulting in the accumulation of toxic substances such as guanidine, and a fall in blood calcium, the fall in calcium being further accentuated by lactation.
- 2) The curative effect of mammary inflation is due to (a) the stimulation of adrenal secretion and the consequent oxidation of toxins, and/or (b) the retardation of the formation of milk and the consequent prevention of further free exchange of calcium from the blood to the milk.
- 3) The preventive value of a restricted withdrawal of milk from the udder after calving is due to this procedure conserving the concentration of calcium in the blood."

*Evidence Supporting the Hypocalcemia Theory*

In May, 1925, Little and Wright (147) first reported that blood serum calcium is low in milk fever and that the severity of the symptoms was in accord with the degree of blood calcium diminution. In mild cases a 20 to 30 per cent reduction in blood calcium was found, whereas in severe cases up to 60 per cent reduction was observed.

The view originally held by Dryerre and Greig that udder inflation stimulated secretion of adrenalin has since been abandoned in the light of the experiments of Auger (13), who was unable to demonstrate any increase in blood pressure after udder inflation.

More recent work by Hayden (106) indicates that guanidine accumulation is not an important factor in the causation of milk fever, as originally suggested by Dryerre and Greig (54). Fish (62) cited the work of Watanabe (260) as evidence of the possible association of guanidine to metabolic changes in milk fever. Hypocholesterolemia, according to Hayden (106), is not a factor in the causation of milk fever, as the values were lower after inflation than before. There was, however, a decrease in the blood cholesterol of parturient cows.

Numerous reports, subsequent to the originals, have been made by Dryerre and Greig, Little and Wright and others which directly or indirectly give support to the essential basis of this theory. Little and Wright (148) point out that 0.5 gal. of colostrum contains as much calcium as is present in the blood at any one time. They attribute much importance to the drain on blood calcium by the secretion of colostrum in the precipitation of the milk fever attack. Dryerre and Greig (55) also reported low blood calcium values in milk fever as further evidence in support of their theory.

In 82 cases of milk fever, Greig (88) reported minimum, maximum and average serum calcium values of 3.00, 7.76 and 5.13 mg., respectively, per 100 ml. No hypocalcemia was found in a study of 81 cases of diseased animals which did not have milk fever (88).

In 15 cases of milk fever successfully treated by udder inflation, Greig (88) demonstrated that the blood calcium rose rapidly to normal and continued to above normal levels after recovery, which resulted when a concentration of about 7 mg. per 100 ml. was reached. It was suggested that inflation of the udder not only stops the secretion of calcium into the milk but may force calcium from the milk back into the blood stream.

Fish (65) reported average milk fever blood plasma values of 3.31 mg. per 100 ml. for calcium and 2.39 mg. per 100 ml. for phosphorus. Phosphorus reached normal after inflation of the udder and rose above normal values in 6 to 8 hr., while calcium was still below normal after 24 to 36 hr.

Niedermier and Smith (170) recently reported data on the blood levels of calcium, phosphorus and magnesium during the recovery period for seven cases of milk fever treated by udder inflation. They pointed out that different cows recovered at different blood calcium levels and suggest that the relative levels of calcium, phosphorus and magnesium may be more important in the symptomatology of milk fever than the level of any one constituent.



Greig (86) showed that a blood picture similar to that of milk fever occurs in "lambing sickness" in ewes and that both udder inflation and calcium gluconate injection were effective as cures.

In 1935, Dryerre and Greig (56) recommended the subcutaneous injection of calcium boro-gluconate as a treatment of milk fever. The nature of this compound is discussed. This treatment reportedly cut down the severity of the heart reactions and decreased the number of relapses due to its slower absorption. The injection of 1 to 2 oz. of calcium boro-gluconate immediately after calving and repeated after 20 hr. was recommended for the prevention of milk fever.

In 1933, Mattick and Little (155) reported on a study of the calcium and phosphorus content of cow's blood. Their findings can be summarized as follows: (1) The calcium and phosphorus content of heifers' blood decreased at parturition. This decrease could not be altered by ultra violet irradiation (one cow). Erf (58), however, reports that blood calcium in cows was elevated by ultra violet irradiation, especially when the skin was oiled with cod liver oil. (2) Parathyroid injections were of doubtful value in preventing or curing milk fever (450 Hansen units as "Paroidin" daily for 4 days before, on the calving date and one day after parturition). (3) Drenching with calcium lactate and  $\text{CaCl}_2$  had no beneficial effect on blood calcium; however,  $\text{CaCO}_3$  plus cod liver oil may have been beneficial in one case. (4) Diffusible calcium was never above 3 mg. per 100 ml. in eight cases of milk fever, whereas total calcium was only once above 6 mg. per 100 ml. (5) Slow intravenous injections of  $\text{CaCl}_2$  solution were specific in curing all cases of milk fever, as had been previously reported by Sjollem (224). (6) Excess amounts of phosphorus in the pastures, fish meal or insufficient lime in feedstuffs seemed to favor the development of milk fever.

Little and Mattick (146) were unable to prevent the decrease in blood calcium at parturition by feeding cod liver oil, although a slight beneficial effect was noticed. A greater percentage drop in diffusible calcium was observed at parturition than in total calcium. Diffusible phosphorus was shown to decrease more than total phosphorus. Phosphorus values returned more rapidly to normal at parturition than did calcium values and the phosphorus values seemed to be higher at parturition in the group fed cod liver oil than in the control group. Methods for both diffusible phosphorus and calcium are described.

Stimulated by the work of Dryerre and Greig and Little and Wright, numerous research workers in all parts of the world reported experiments dealing with various phases of the milk fever problem.

Sjollem (224, 225), of the University of Utrecht, reported low values for both serum calcium and phosphorus in 40 milk fever cases and recommended 300 to 400 ml. of 10 per cent  $\text{CaCl}_2$  as a cure. He stressed the importance of upset electrolyte balance in the etiology of milk fever as a result of determining the calcium, magnesium, potassium, chloride, phosphorus, pH and alkaline reserve on blood serum and of total sugar, glucose, urea and total acetone in whole blood.

Fish (63, 64, 65, 66, 67) presented evidence against the hypoglycemia theory and in support of the hypocalcemia theory. He pointed to the disturbed nitrogen metabolism and hyperglycemia of milk fever cows as an indication of defec-

tive oxidation in the body and the presence of a series of phenomena in milk fever. Decreased calcium and increased guanidine indicated that the parathyroids were involved. He brought attention to the ratio of calcium to phosphorus in milk fever (64). The Ca:P ratio in the blood of milk fever cows is 1.9; in normal cows the Ca:P ratio is 2.31.  $\text{Ca} \times \text{P}$  in milk fever cows is 8.18 and in normal cows is 50.08. He suggested that hyperglycemia seen in milk fever may be due to disorganization of the hexose phosphate constituent in muscle due to the low blood phosphorus, but that it is of no particular significance so far as the etiology of milk fever is concerned.

In discussing Harding's theory of anhydremia, Fish (66) indicated a 14 per cent increase in red blood cells and a 7 per cent decrease in serum protein in milk-fever cows. Fish (67) and Gould (83) stressed the importance of diet in preventing milk fever. Phosphorus was believed to be just as important as calcium in the diet. Calcium glycerophosphate with glucose was recommended for intravenous treatment. Appleby (10) recently has recommended the use of calcium boro-gluconate-saccharate as a treatment for milk fever.

A comprehensive review of the knowledge of milk fever in 1932 is given by Sjollem (226). The disease is discussed from the standpoint of occurrence, metabolism, etiology and therapy.

From 1930 to 1932 Sjollem *et al.* (227, 228, 229) and Seekles *et al.* (218, 219, 220, 221) published a series of papers which added materially to the knowledge of the etiology of milk fever.

Sjollem (227, 228) drew comparisons between the blood picture of normal cows and those with milk fever and grass tetany, as follows:

	Ca ion (mg. %)	Inorg. P (mg. %)	Mg (mg. %)	Total Ca (mg. %)
Milk fever	0.44	2.16	2.19	4.35
Grass tetany	1.18	4.33	0.46	6.65
Normal cattle	1.65	4.57	1.66	9.35

Ca:Mg ratio equals 2 in milk fever and is 14.6 in grass tetany. The diffusible calcium level of the blood paralleled the severity of the symptoms more closely than did total serum calcium. This also was reported by Seekles *et al.* (220).

Carlstrom and Ecklund (Huttyra *et al.*, 126) injected sodium oxalate into cows, causing hypocalcemia and symptoms of milk fever.

Sjollem *et al.* (219) also injected sodium oxalate into cattle and observed the symptoms. Serum calcium was greatly reduced and death with clonic convulsions occurred when levels lower than 4.0 mg. per 100 ml. were reached. Sodium citrate did not lower the total serum calcium. He concluded that milk fever is not simply the result of lowered serum calcium but that this is only part of a more general disturbance of metabolism. Similar experiments using sodium citrate were carried out by Petersen *et al.* (180) with the same results and conclusions.

Blosser and Smith (29) in a recent publication have shown a significant cor-

relation between blood serum citric acid and calcium during the period of parturition. A marked drop in blood citric acid occurred at parturition in both normal and milk fever cows.

Sjollema, *et al.* (218), in a series of papers on the mode of action of calcium, noted that after injection of sodium oxalate, serum calcium was at its lowest within 10 min. The reduction was relatively less for the ultrafilterable than for the "bound" fraction. A residue of 3 to 4 mg. per 100 ml. exists which is not precipitated by large excesses of oxalate. Calves were less sensitive to oxalate than older cattle. When serum calcium is lowest, heart symptoms may occur. Ataxia, paralysis and tremor occur after the lowest point is passed when serum calcium is again rising.

Magnesium sulfate was injected into calves to produce magnesium narcosis which, according to Kloubok (134), Pribyl (190) and Schulhof (212), is the cause of milk fever. The Ca:Mg ratio in the injected calves was similar to that found in milk fever, and  $\text{CaCl}_2$  injections were curative in both cases. Hayden (107) claims the slight increase in serum magnesium in milk fever is of no significance, based on the normal values of Eveleth (59). Meltzer and Auer (157) have demonstrated an antagonism between serum calcium and magnesium. In studying the effects on the heart of injected  $\text{CaCl}_2$  and  $\text{MgCl}_2$  in milk-fever and grass-tetany cows, they showed that the effect of each injected separately was the same, resulting in increased rate and disturbed rhythm. When injected as a mixture, no toxic symptoms resulted. In 30 cases of milk fever injected with a mixture of  $\text{CaCl}_2$  and  $\text{MgCl}_2$ , some of which had extremely low ionized calcium and were likely to develop heart block, no bad effects were observed. This work later was confirmed by the same authors (229).

Waife (256) in studying "Partial heart block in hyperparathyroidism" describes incomplete heart block during hypercalcemia which may not disappear completely when serum calcium returns to normal or falls below normal. The electrocardiogram showed long Q-T. intervals when serum calcium was below 8 mg. per 100 ml. Spörri and Raggenbass (236) also have studied the effect of calcium and air insufflation therapy of milk fever on the electrocardiogram. They found that after calcium injections, the electrocardiogram returned to normal. Hypocalcemia had a marked effect on the heart musculature.

In a study of the effect of blood changes on the tone of the autonomic nervous system during pregnancy and parturition in normal cattle, Seekles and Sjollema (221) reported that serum calcium and phosphorus decreased about 4 days before parturition, reaching a low point just before or shortly after parturition. Serum magnesium was in inverse relation to calcium and phosphorus. Potassium reached a minimum several days postpartum. These marked fluctuations are attributed to "lability of the regulatory mechanisms." Blood sugar increased in seven cases at parturition. No change in "rest nitrogen" was observed. The vagus tone of the heart increased as pregnancy progressed, due to the rise in the K:Ca ratio. An atypical case of milk fever was believed to have been cured by the injection of parathyroid extract. The total calcium was lower after recovery but the ionized calcium was doubled. Seekles *et al.* (220) reported that  $\text{CaCl}_2$

will raise the total calcium in the blood and that parathyroid injections raise the ionized calcium. Both were alleged to cure the symptoms.

Palmer *et al.* (174, 175) had previously reported a decrease in both calcium and phosphorus in the blood of normal cattle at parturition, as had Godden and Alleroft (80). Phosphorus apparently is capable of wide variations without affecting the health of the cow. Normal values and variations in milk fever for serum calcium, phosphorus and magnesium also were reported by Alleroft and Green (6). Alleroft and Godden (5) reported increases in magnesium at parturition or 24 hr. thereafter in normal cattle. Godden and Duckworth (81) reported values for serum magnesium, calcium and calcium partition in the blood of cows at parturition and in milk fever. A steep fall in total serum calcium preceded the acute symptoms of milk fever before and at calving. The symptoms arise when there is a simultaneous fall in both adsorbable calcium complexes and the non-adsorbable calcium ("protein-bound" calcium plus calcium ion). The fall in adsorbable calcium complexes accounts for the major part of the fall in total serum calcium in milk fever. Serum magnesium and calcium rise together during the recovery stage of milk fever.

As a possible explanation of the low serum phosphorus values in milk fever, these authors suggested that this would be expected when a fall in the ultrafilterable calcium complex occurs. Benjamin and Hess (25) showed that, in the low-phosphorus type of rickets, the adsorbable calcium complexes were below normal and that this was coincident with low serum inorganic phosphorus. Benjamin and Hess (25) also showed that ultrafilterable calcium complex contained calcium, phosphate and bicarbonate ions. Thus, any decrease in the amount of ultrafilterable calcium complex would result in a corresponding decrease in the inorganic phosphorus, unless more phosphorus were secreted into the blood stream to make good this deficit. Therefore, instead of the normal inverse relationship between calcium and phosphorus in the blood, both calcium and phosphorus fall together in milk fever, caused by the tremendous reduction in the  $(\text{Ca})_x(\text{Po}_4)_y(\text{HCO}_3)_z$  complex resulting from colostrum formation. The failure to mobilize calcium in milk fever, whether directly or indirectly dependent on the parathyroids, or not, thus would account for the low phosphorus in milk fever.

Frei and Demmel (70) reported very little variation in serum composition in lactating and pregnant cows. The changes in milk fever were attributed to disturbances in the endocrine and vegetative nervous system associated with parturition, rather than the onset of milk secretion. These authors linked the vegetative nervous system, metabolic centers in the medulla, endocrine and blood ionic equilibrium, and considered a disturbance at any one point to affect the whole chain.

Robinson and Huffman (201) reported that the blood bicarbonates are lowered after parturition.

Hayden (107) reported low lipid, total acid-soluble and total phosphorus in milk fever. In later papers, Hayden (108) and Sampson and Hayden (205) showed that the  $\text{CO}_2$  capacity of the blood is lowered in milk fever and acetonemia and that blood ketones are increased.

Hart *et al.* (102) reported that HCl fed to cows increased the urinary calcium excretion and also the absorption of calcium.

Wilson and Hart (272), in a detailed study of blood constituents, presented evidence that anhydremia is not an important factor in milk fever due to the slight lowering of protein values in milk fever as compared to normal calvings. A greater decrease in serum calcium and phosphorus occurred at parturition in older cattle than in first-calf heifers. Blood phosphorus and calcium were found to decrease sometime during the first 3 days postpartum. Additional evidence was presented showing that serum calcium deficiency is the essential factor in milk fever.

In a study of the plasma phosphatase, these same authors found that there is a decrease from 3 wk. pre-freshening to near the time of calving when it tends to increase, falling again during the 3 wk. postpartum. Milk phosphatase was higher than plasma phosphatase, being especially high at the end of lactation, as compared with beginning lactation in heavily producing cows.

Allcroft and Folley (4) indicated that serum phosphatase varies widely among individuals but is rather constant in the same individual over long periods. Pregnancy raises serum phosphatase activity but there was no correlation with stage of lactation. There was no correlation between milking capacity and phosphatase activity.

### *Predisposing Factors*

Ever since milk fever first was mentioned in the literature, numerous conditions have been associated as predisposing factors. It immediately was recognized that age was one of these. First-calf heifers were seldom, if ever, known to have milk fever and it was recognized that from 5 to 10 yr. of age, the period of highest production, are the years in which milk fever is most likely to occur. Metzger and Morrison (159) and Henderson (113) presented data confirming this observation.

Milk fever usually is thought to be associated with "deep milking qualities" or heavy producing cows. However, this is not a hard and fast rule, as numerous cows which were not outstanding producers have been known to have milk fever.

Hibbs (117) has presented evidence that milk-fever cows produce no more colostrum than cows that freshen normally and that the ash and calcium content of the colostrum of milk-fever cows is no higher than that of cows freshening normally.

Heavy feeding during the dry period has been cited as a factor leading to milk fever. A 50 per cent decrease in occurrence was noted during World War I in Europe, according to Gruter (89), when feed was scarce.

Scottish farmers are said to feed straw and turnips during late pregnancy as a preventive measure. However, Maguire (153) stated that the system of "steaming," or heavy feeding of grain high in carbohydrate and protein, used by English farmers in late pregnancy is said to decrease milk fever incidence and raise milk production. This, therefore, is a controversial question.

Confinement sometimes is associated with milk fever, although there is little basis for this idea. Albrecht (2), in 1904, observed that milk fever disappeared from a herd when cows were allowed to calve out in the open instead of in closed stalls.

Diets high in phosphorus and/or deficient in calcium have been cited as the cause of milk fever by Gould (83), Fish (64), Little *et al.* (144, 155) and Götze (82), to name a few.

Götze (82) stated that gestation and lactation test, to the limit of endurance, the visceral nervous system if important mineral constituents are lacking or are in excess. He was of the opinion that success in research on milk fever is not to be found in research on therapeutics but in appropriate nutrition which will keep the visceral nervous system balanced. He stressed the importance of mineral balance of the soil on which roughage is grown and suggested that the composition of roughage and concentrates should be known and balanced with supplements.

Greig (91), in the discussion of the paper by Götze, pointed out that milk fever is an acute hypocalcemia brought on by sudden onset of lactation (easy parturition, no mastitis, etc.). The parathyroids being quiescent, the tissue calcium cannot be mobilized. He stated that colostrum contains more calcium than the blood at any one time. Therefore, he believed that balanced intake is not of major importance in the etiology of milk fever.

While diet may be a factor, the present understanding of the etiology of milk fever does not favor this concept, especially in view of the rapidity of the changes in blood calcium and phosphorus both in the onset and during the recovery period of milk fever.

Krøgoe and Petersen, according to Huttyra and Marek (126), claimed that more cases occur when the barometer is falling than when it is high. This was denied by Stenius (Huttyra *et al.*, 126) who observed 54 per cent when the barometer was falling, 9 per cent when stationary, and 37 per cent when rising. Stenstrup (Huttyra *et al.*, 126) reported only 15 out of 94 cases of milk fever occurred during or following a fall in atmospheric pressure. Heately and Dryerre (Huttyra *et al.*, 126) showed no correlation with climatic conditions in prolonged observations. Hallgren (96) and Morin (162) also denied the claim that milk fever is more likely to occur during a falling barometer. Likewise, Hibbs (117) has presented evidence that there is no correlation between barometric pressure and the onset of milk fever. Morin (162) stated that milk fever rarely occurs in premature calvings but frequently in overtime cases and when a large calf is born.

Cows that have one attack are likely to repeat at subsequent parturitions. This indicates impairment of resistance, according to Huttyra and Marek (126), rather than that the cow is rendered more susceptible by the first attack.

Considerable data indicates a breed difference in susceptibility to milk fever. Henderson (113) and Metzger and Morrison (159) indicated that cows of the Jersey breed are many times as likely to develop milk fever as cows of the same age group in other breeds. Hibbs *et al.* (119) confirmed this observation. How-

ever, Hibbs *et al.* (119) were unable to show a seasonal variation in the incidence of milk fever, as was shown by Henderson (113) and Metzger and Morrison (159), who reported a higher milk fever incidence in the winter months than in the summer months. Henderson (113) reported 4.07 per cent of susceptible parturitions in the months of May to September and 13.35 per cent in the months of October to April. Evidence presented by Smith and Blosser (232) also indicates that there is no seasonal influence on milk fever incidence.

In weighing all the experimental evidence that has been reported on the various phases of the milk fever problem, the theory of Dryerre and Greig seems to come the nearest of all theories to explaining the cause of the disease. However, numerous questions are as yet unanswered. One of these is whether or not deficiencies in the feed result in the upset mineral metabolism seen in milk fever. As Sjøllema (226) points out, mineral deficiencies in the feed are the cause of such disorders as aphosphorosis, rickets, sterility and border-line conditions which only develop acutely when some other agent is operative.

Milk fever symptoms do not develop in conditions such as nephritis or during recovery from parathyroidectomy in dogs, although the serum calcium level is comparable to that seen in milk fever. The difference in symptoms between milk fever and nephritis possibly is explained on the basis of the ionized calcium level, according to Sjøllema.

Furthermore, milk-fever symptoms are not seen in aphosphorosis of cattle in South Africa, although the blood levels are comparable insofar as phosphorus is concerned. However, the possible effects of diet, repeated lactations and maturity of the animal on the supply of readily available stores of calcium and phosphorus in the bones must not be overlooked in this connection.

The fact that the onset and recovery from milk fever is so sudden, regardless of treatment (calcium injection or udder inflation), is further indication that a dietary deficiency may not be a primary etiological factor, except as it may influence the supply of readily available calcium and phosphorus stored in the tissues.

It seems that the drain on the blood calcium for the production of colostrum is not the primary cause of milk fever since, as shown by Hibbs (117), in normal parturitions an equal drain on blood calcium and phosphorus for colostrum production is experienced which results in but a slight decrease in the blood levels. It is more likely that the cause is the inability of the regulatory system to meet this sudden demand through mobilization, as suggested by Greig. The cause of the failure of the blood calcium regulatory mechanism to meet the demand is yet to be discovered. It is reasonable to believe, however, that colostrum production does act as a "precipitant" in the chain of events leading to the onset of milk fever.

Based on differences in calcium values between the jugular and mammary veins, Hallgren (96) attributed the lowering of serum calcium in milk fever to its excretion in the colostrum.

The recent work of Smith *et al.* (232, 233) is of great interest in this connection. They have shown that no decrease in milk fever incidence resulted from

prepartum milking, nor did any increase in incidence result from complete milking as compared to partial milking after parturition. Niedermier *et al.* (169) have shown further that the blood serum calcium and fat decrease less at parturition in mastectomized cows than in cows whose udders are intact. Serum magnesium did not increase at parturition in the mastectomized cows as it does in normal cows. Serum phosphorus levels showed a drop in both mastectomized and normal cows.

The answer to the question of how equilibrium is restored so rapidly following treatment is not apparent from experimental evidence presented to date.

#### *Blood Magnesium Level as Related to Milk-fever Symptoms*

The central nervous system apparently is involved in the appearance and disappearance of the symptoms of milk fever. Seekles and Sjollem (218), as previously mentioned, have shown that the comatose condition produced in calves after magnesium injection is cured immediately by calcium injection. In experiments with rabbits this effect took place only if the corpus striatum was intact (Yamawaki, 274).

Barker (16) recognized three types of hypocalcemia, depending upon the level of serum magnesium. The symptoms seen varied from extreme nervousness and tetany when magnesium was low to a comatose condition when magnesium was high. He recommended a 25 per cent  $\text{MgSO}_4$  solution be given along with calcium gluconate as a treatment for milk fever.

Neuwirth and Wallace (168), working with dogs, showed that a profound coma resulted when the serum magnesium was raised to 18 to 21 mg. per 100 ml. by injecting  $\text{MgSO}_4$ . Greville and Lehman (93) have shown an antagonism between magnesium and calcium in muscle. Meltzer and Auer (157) showed that dogs and monkeys could be brought out of magnesium narcosis by calcium injections.

Sjollem *et al.* (218) found that calves injected with  $\text{MgSO}_4$  so as to produce a comatose condition were cured by the intravenous injection of  $\text{CaCl}_2$ .

Moore and Wingo (160), in investigating the blood level of magnesium ion in relation to its lethal, anesthetic, analgesic and antitetanitic effects, found that in cats under nembutal anesthesia the fatal blood level of magnesium was 24.7 mg. per 100 ml. In dogs under similar conditions, the lethal level was 24 to 32 mg. per 100 ml. The fatal blood level of magnesium increased somewhat when the degree of anesthesia was decreased. When calcium was injected concurrently with magnesium, the fatal level of magnesium increased to 65.8 mg. per 100 ml. Low doses were found to offset tetany due to low calcium.

An interesting sidelight is the report of Suomalainen (241) who reports that during hibernation in the hedgehog, the serum magnesium increases from a normal of 3 mg. per 100 ml. to 6 mg. per 100 ml., while the serum calcium remains constant at from 9.8 to 10.6 mg. per 100 ml. Thus, the Ca:Mg ratio during hibernation is not far from that seen in milk fever in cows.

It was apparent from the results of Hibbs *et al.* (117, 120) that the anesthetic effect of magnesium is due to the ratio of calcium to magnesium, regardless of



whether the serum calcium comes down to meet the magnesium, as in milk fever or when sodium oxalate is injected, or the serum magnesium goes up to meet the serum calcium, as when magnesium salts are injected.

The increase of serum magnesium preceding milk fever is not readily explained. Possibly it is due to a compensatory measure elicited by the fall in serum calcium, or it may be a protective antitetanic phenomenon. However, it seems reasonable to conclude that the relatively high serum magnesium accounts for the lack of tetanic symptoms in typical milk fever and the comatose condition which usually is seen in spite of the low blood calcium level.

#### *Parathyroid—Vitamin D—Blood Calcium Relationships*

The role of the parathyroids presents a challenging phase for future research in milk fever. Morgan *et al.* (161) have shown that dogs on a vitamin D-deficient diet showed a smaller response to parathyroid injections in serum calcium than normal dogs and that on low-calcium diets an increase in serum calcium resulted only after the first injection of parathyroid extract.

Campbell and Turner (35) reviewed the literature pertaining to the relation of parathyroid activity to calcium metabolism. Collip (41) was one of the first to demonstrate the function of the parathyroid glands in the regulation of blood calcium.

Seekles *et al.* (220) claim that milk fever was cured in a few cows by parathyroid extract injections.

Jaffe *et al.* (128) suggest that, in hyperparathyroidism, calcium is rapidly resorbed from the sites of rapid bone growth. Thus, young rapidly-growing animals respond to injections of parathyroid hormone more readily than do older animals. This may be the explanation of, or at least a factor in, the common observation that cows usually do not develop milk fever until after the second parturition or after the period of rapid bone growth. Bodansky and Jaffe (30) state that the hypocalcemia following hypercalcemia induced by parathormone injection is due to calcium redeposition in the bones. Holtz *et al.* (122) confirm this observation.

Gerschman (75) and Patt and Luckhardt (177) report a reciprocal relationship between parathyroid activity and the blood serum level of calcium. Stoerk and Carnes (239) have found a linear inverse relationship between parathyroid volume and serum calcium levels between 7.3 and 11.9 mg. per 100 ml. in the rat.

Taylor *et al.* (242, 243) show that the effects of excessive doses of irradiated ergosterol upon calcium and phosphorus metabolism of the dog run closely parallel with those resulting from parathyroid overdosage. Species showing a high resistance to the toxic action of irradiated ergosterol also are correspondingly tolerant to the action of parathormone. These authors favor the hypothesis that vitamin D increases serum calcium primarily by its effect on parathyroid tissue.

Taylor *et al.* (244) have shown further that dogs made tolerant to parathyroid were resistant to large doses of irradiated ergosterol (400,000 I.U./kg. for three doses).

A limited amount of work with cows done by Hibbs *et al.*, some of which has

been reported (118), showed that a response of 1.5 to 2.0 mg. per 100 ml. in serum calcium and phosphorus resulted 15 to 20 hr. after injecting 2,000 to 3,000 units of "Paroidin" subcutaneously. A transitory rise and fall in serum magnesium resulted before the peak in serum calcium was reached, which is in accordance with the results of Scholtz (211) working with dogs. Results obtained in treating cows with early milk fever symptoms with parathyroid hormone were not encouraging. All had to be injected with calcium due to severity of symptoms before any response in blood calcium was obtained. Indications are that the failure to mobilize calcium from the skeletal reserves in milk fever may be due either to a failure of the parathyroid glands to secrete sufficient hormone or to a situation in which sufficient hormone is secreted but is rendered temporarily inactive by some metabolic condition in the tissues at parturition.

As previously mentioned, Hayden (108) and Sampson and Hayden (205) reported that the  $\text{CO}_2$ -combining power of the blood is lowered in milk fever. Craig *et al.* (46, 47, 48), on the other hand, attribute milk fever to alkalosis accompanied by increased  $\text{CO}_2$  capacity of the blood.

It is interesting to speculate on the statement of Voinar and Babkin (255) that the narcotic anoxemia following injection of oxalic acid into narcotized dogs results in increased production and excretion of oxalic acid by the body. The possibility exists that some factor such as this may be responsible for inactivating the parathyroid hormone or tying up serum calcium at parturition. However, no conclusive evidence has been presented in support of this theory.

In connection with the possible relation of the alkaline reserve to milk fever, the work of Lecoq (138) is of interest. He has shown that in guinea pigs the intravenous injection of calcium pantothenate along with thiamin, riboflavin and adenine caused a marked increase in the alkali reserve comparable to the injection of  $\text{NaHCO}_3$ . Calcium pantothenate alone gave no response and when omitted the others gave no response.

Chauchard *et al.* (37) have shown that neuromuscular excitability resulted when alkalosis was induced by injecting  $\text{NaHCO}_3$  (12.5 mg. three times a week) into rats. These disturbances were prevented if a simultaneous but separate dose of 800 U.S.P. units of vitamin  $\text{D}_2$  in glycerol-alcoholic solution was injected intraperitoneally. When vitamin  $\text{D}_2$  was given with  $\text{NH}_4\text{Cl}$ , it increased the neuromuscular signs of acidosis provoked by it.

Gineste (78) presents histological evidence that the adrenal medulla action is increased by vitamin D. It is suggested that this may account for the hypertensive action of vitamin D on the whole organism.

These observations are of interest in that they shed some light on the mode of action of vitamin D in the animal body. In future research it appears that the effect of the alkaline reserve in relation to vitamin D and the parathyroid hormone might well be taken into consideration.

Sato (207) reports the presence of a substance in the blood of the mammary vein of the cow which, when injected into rabbits, has the ability to lower serum calcium.

Numerous reports such as those of Johnston (131), Robertson (195, 196, 197,

198, 199), Hunter (124), Joh (130) and Logan *et al.* (149), show that the thyroid hormone has an anabolic effect on Ca metabolism, manifested by increased calcium excretion and lowered serum calcium. In hypothyroidism the converse is true.

### *Prevention*

The important role of vitamin D in the metabolism of calcium and phosphorus has been recognized for many years. Greig (88), Sjollem (226) and Little and Mattick (146) have suggested that vitamin D might prove beneficial as a preventive in milk fever.

In 1930, Greig (87) reported the successful treatment of 32 cases of milk fever by the intravenous injection of calcium gluconate. This discovery revolutionized the treatment of milk fever, as it was extremely effective and removed the hazards of udder inflation. In the same year, Greig (88) reported the results of his studies on the prevention of milk fever. He suggested vitamin D administration before parturition as a possible preventive measure. He fed 50,000 ostelin units (30,120 U.S.P. units) of vitamin D daily to one cow for a period of 3 wk. An increase in calcium resulted which persisted for 9 days and then returned to normal despite vitamin D administration. The serum calcium level fell below normal when vitamin D was stopped. This cow later had milk fever. The addition of calcium to the blood 5 days before parturition also was suggested (88) as a preventive measure, although little evidence was presented to support the suggestion.

Greig (92) also suggested that calcium gluconate be injected subcutaneously immediately after parturition as a prophylactic. Greig (90) reported favorable results using intramuscular injections of calcium gluconate to cure milk fever.

Hess *et al.* (116) reported no increase in the blood calcium of cows receiving 60,000 units of vitamin D daily. However, vitamin D is known to have calcemic value. Campbell and Turner (35) reported experiments in which the calcemic properties of various vitamin D preparations were tested on lactating goats. Increases in blood calcium and phosphorus were observed when A.T. 10 (Hytakerol) was injected in doses of 80 ml. over a period of 4 days. This is equivalent to 32,000,000 I.U. of vitamin D. A.T. 10 has a calcemic value twice that of calciferol. These authors also suggested the possibility of utilizing the calcemic properties of vitamin D to buffer the sudden demands for calcium mobilization and increased parathyroid activity at parturition and beginning lactation, thereby lessening the danger of milk fever.

Campbell and Turner (35) confirmed and extended the observations of Bastenie and Zylberzac (20) that high doses of A.T. 10 were toxic to rats and that parathyroid activity actually is suppressed by excessive doses. Blood calcium levels of 18 to 20 mg. per 100 ml. were found in the toxic rats. Hess *et al.* (116) had observed no toxic symptoms in cows fed as much as 1,500,000 units of vitamin D daily.

Campbell and Turner (35) showed that vitamin D administration causes a decrease in the animal's own parathyroid activity when fed for long periods of time. In other words, there is a strong tendency for the animal to maintain a

normal blood calcium level and if this is accomplished with supplemental vitamin D or high calcium rations, then the cow's own parathyroid gland is not needed and lapses into a hypoactive state. Therefore it seems reasonable that if vitamin D were fed in large amounts for too long a time, the animal's own calcium-mobilizing power may be suppressed to the point that the beneficial or supplemental action of the vitamin D feeding on calcium mobilization would be nullified.

Under these conditions when parturition and beginning lactation occur, the cow would be in no better condition to meet the sudden demand for calcium than if no vitamin D were fed. Thus, it is apparent that in the use of vitamin D as a possible preventive in milk fever, care should be exercised in selecting a dosage which would be non-toxic and one which would supplement rather than depress parathyroid activity.

In earlier experiments conducted by Hibbs *et al.* (119, 120) no reduction in milk fever incidence was obtained from feeding from 1 to 5 million units of vitamin D daily for from 2 to 4 weeks prepartum. Although blood calcium was increased considerably at the higher levels of vitamin D feeding just prior to parturition, the increase was nullified within 24 hr. postpartum in normally freshening cows.

In more recent experiments, a part of which has been reported by Hibbs *et al.* (118), massive doses of vitamin D have been administered limiting the dosage to the period of a few days just prior to parturition. It was reasoned that by timing the dosage so that the peak in the blood calcium level was reached just prior to parturition, the vitamin D would have the maximum supplemental action without possible parathyroid suppression due to prolonged elevated blood calcium. The results to date based on the blood changes in calcium, phosphorus and magnesium and on the incidence of milk fever have been most gratifying. It is believed that an effective, practical, preventive measure for milk fever can be based on these findings.

#### SUMMARY AND CONCLUSIONS

The history of milk fever (parturient paresis), traced from the time reports first began to appear in the literature (about 1793) indicates that this metabolic disturbance has been associated with the development of the dairy cow for high milk production. Some thirty theories of the etiology of milk fever have been advanced through the years. As new scientific knowledge and techniques have developed, the false hypotheses gradually have been eliminated so that a complete understanding of the fundamental basis of milk fever now awaits only the proof of certain points in the present concept which now are based on circumstantial evidence.

The evidence indicates that the lowered blood calcium in milk fever is due to the failure of the blood calcium regulatory mechanism to mobilize calcium from the tissue reserves rapidly enough to equal the withdrawal of calcium from the blood into the udder secretions.

If it is assumed, as the evidence indicates, that the parathyroid hormone is the primary regulator of blood calcium, its failure in milk fever may be caused

by either parathyroid inadequacy resulting in the lack of sufficient hormone secretion or by the presence of some metabolic condition in the tissues at parturition that renders the parathyroid hormone temporarily inactive. It would seem, therefore, that further work on the function of the parathyroid glands at parturition must be done before the fundamental basis of milk fever can be proven.

Any preventive measure must be aimed at eliminating the precipitous fall in blood calcium at parturition. Vitamin D in large amounts just prior to parturition offers the most encouraging possibilities for prevention. However, it is not known as yet whether the vitamin D acts to increase blood calcium *per se* or *via* the parathyroid glands.

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## TOCOPHEROL, CAROTENOID AND VITAMIN A CONTENT OF THE MILK FAT AND THE RESISTANCE OF MILK TO THE DEVELOPMENT OF OXIDIZED FLAVORS AS INFLUENCED BY BREED AND SEASON

VLADIMIR N. KRUKOVSKY, FRANK WHITING<sup>1</sup> AND J. K. LOOSLI

*New York State College of Agriculture, Cornell University, Ithaca*

The work at this station dealing with deteriorative processes in milk and milk products involving ascorbic acid oxidation have shown that a relationship exists between the tocopherol content of milk fat and the ability of milk to resist the reactions which produce the oxidized flavors, and that both the tocopherols and the stability of milk are influenced by the type of hay and pasture fed to the cow (6, 7, 8). It has first been postulated and then shown on cream (7, 9) that the increase in the anti-oxidant activity of fat as determined by the tocopherol method resulted in the inhibition of development of oxidized flavors associated with deterioration of unstable lipids of the fat globule membrane and in the prolongation of the storage life of fat as determined by the re-emulsification test (5, 9).

Consequently, a study was made to determine the normal tocopherol content of milk produced by different breeds of dairy cows throughout the season and under standard feeding conditions commonly employed at the Cornell Station.

### EXPERIMENTAL

Samples of morning milk were collected from cows of Holstein, Brown Swiss, Jersey and Guernsey breeds in the Cornell University Herd, in October, 1947, toward the end of pasture season; in March, 1948, after 5 mo. of barn feeding; and again in July, 1948, following 3 mo. of pasture feeding. Milk was pasteurized at 61.6° C. for 30 min., and the stability of milk was determined on the basis of its ability to resist the reactions which produce the oxidized flavors during 7 days storage at 0 to 5° C. A part of this milk was separated by gravity creaming. The cream was churned and the butter obtained was melted and centrifuged clear and the fat was analyzed for the fat-soluble vitamin content. Vitamin A, carotenoids and tocopherols were determined using Koehn and Sherman (3) and Quaife (10) methods, respectively.

### RESULTS

The average values for tocopherols, carotenoids and vitamin A content of dif-

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<sup>1</sup> Now at Dominion Expt. Station, Lethbridge, Alberta, Canada.



ferent milk fat samples are presented in table 1. The data show large variations

TABLE 1  
*Tocopherol, carotenoid and vitamin A of the milk fat as influenced by breed and season*

Breed	Date	No. of cows	Quantities per 100 g. of fat			
			Total tocopherols	Carotenoids	Vitamin A	Total vitamin A
			( $\mu$ g.)	( $\mu$ g.)	( $\mu$ g.)	(I.U.)*
Holstein-Friesian	10/24/47	18	2253 $\pm$ 822	504 $\pm$ 249	546 $\pm$ 145	3020
	3/26/48	20	2011 $\pm$ 341	290 $\pm$ 109	398 $\pm$ 86	2076
	8/ 1/48	13	2492 $\pm$ 369	774 $\pm$ 276	908 $\pm$ 152	4922
	Av.	51	2220	489	580	3135
Brown Swiss	10/24/47	11	2860 $\pm$ 656	785 $\pm$ 221	703 $\pm$ 146	4120
	3/26/48	9	2149 $\pm$ 487	341 $\pm$ 165	383 $\pm$ 47	2100
	8/ 1/48	13	2567 $\pm$ 563	1019 $\pm$ 309	859 $\pm$ 131	5134
	Av.	33	2550	756	677	3968
Jersey	10/24/47	5	3036 $\pm$ 498	1236 $\pm$ 358	578 $\pm$ 123	4372
	3/26/48	4	1905 $\pm$ 361	341 $\pm$ 108	301 $\pm$ 26	1772
	8/ 1/48	7	2740 $\pm$ 508	1370 $\pm$ 109	631 $\pm$ 166	4807
	Av.	16	2623	1070	532	3911
Guernsey	10/24/47	9	3164 $\pm$ 462	1583 $\pm$ 237	381 $\pm$ 120	4162
	3/26/48	7	2329 $\pm$ 343	772 $\pm$ 169	312 $\pm$ 109	2534
	8/ 1/48	12	3346 $\pm$ 692	2484 $\pm$ 512	663 $\pm$ 259	6792
	Av.	28	3033	1766	485	4883
Av. of all breeds	10/24/47	43	2763	887	555	3698
	3/26/48	40	2087	391	370	2131
	8/ 1/48	45	2779	1394	785	5463
Grand Total Av.		128	2533	910	578	3828

\* 0.6 micrograms of the carotene and 0.25 microgram of the vitamin A are equal each to 1 I.U. of vitamin A.

in tocopherol, carotenoid and vitamin A content of the fat between individual cows of the same breed, even though they were fed the same rations. There also are wide variations between different breeds and between seasons. As an average, the samples of fat obtained from Guernsey milk were higher in tocopherols, carotenoids and total vitamin A content than the milk fat of any other breed, and this difference held to some extent for any season, as it is shown in table 1.

Holstein milk fat samples were uniformly lower in tocopherols and carotenoid content, and Brown Swiss and Jersey samples were intermediate. During the pasture season the fat samples were 24 per cent higher in tocopherols, 55 to 71 per cent higher in carotenoids and 42 to 60 per cent higher in total vitamin A activity than during the barn feeding. As an average (grand total), the samples of fat contained 2533  $\mu$ g. of tocopherols and 3828 I.U. of total vitamin A activity per 100 g. of fat. The total vitamin A activity was found to be only slightly below that reported by the U.S.D.A. butter survey committee (12), (14,098 I.U. and 15,529 I.U. per pound of butter, respectively).

The relationships between tocopherol, carotenoid and vitamin A content of the fat from four breeds of dairy cows as affected by both pasture and barn feeding are presented in figure 1. A highly significant correlation has been

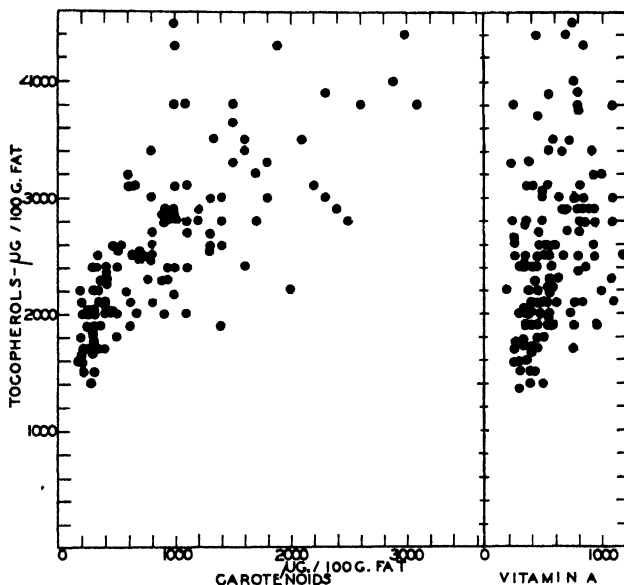


FIG. 1. The relationship between the tocopherol and carotenoid and vitamin A content of the milk fat from four breeds of dairy cows as affected by both pasture and hay feeding (128 samples of milk).

found between tocopherol and carotenoid content of the fat (+ 0.69, + 0.63 and + 0.68 for the three sampling periods, respectively). No significant correlation could be shown between tocopherol and vitamin A.

The data in figure 2 show the per cent distribution of tocopherols in samples of stable and unstable milks as affected by both pasture and barn feeding. They show again as before (7) that the stability of the fresh pasteurized milk was improved when its tocopherol content was increased to 3,000 µg. and above per 100 g. of fat.

#### DISCUSSION

Although the data presented in figure 2 were rather conclusive in showing that the anti-oxidant activity centered in the fat phase of the milk plays an important part in the inhibition of oxidized flavors associated with deterioration of the unstable lipid components (9) of the milk system, nevertheless it is necessary also to consider the related effect of the additional factors. This is evident from the observations showing that some of the samples of milk of low tocopherol content did not develop the oxidized flavors during 7 days storage at 0 to 5° C. This fact can be explained by the assumption that the type and quality of the roughages fed to the cow, together with the physiological response of the

cow may determine not only the fat constants and the assimilation and deposition of tocopherols into the milk fat, but also the catalytic properties of the milk with respect to its natural ability to promote ascorbic acid oxidation. This particular factor can be responsible either for too rapid or too slow rate of oxidation of ascorbic acid, thus delaying the onset of the coupled reactions which produce the oxidized flavors. In this connection it should be noted that the rate of ascorbic acid oxidation is an important factor in the promotion or retardation of oxidized flavors in milk (2, 4). Furthermore, the presence of more readily oxidizable substances than the unstable lipids of the milk may result in a selective and stepwise oxidation of the respective components of the milk system. In such a case, the deterioration of unstable lipids might be postponed or not have taken place at all, depending on the availability of ascorbic acid. Likewise, an

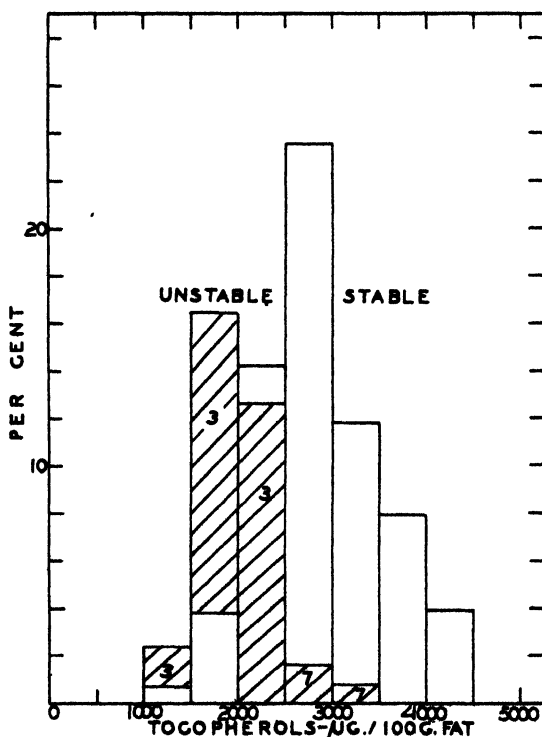


FIG. 2. The distribution of tocopherols in 128 samples of stable and unstable natural milks as affected by both pasture and hay feeding (seasonal variations). The numbers (3) and (7) indicate the days within which the oxidized flavor developed in unstable milk.

increase in the anti-oxidant activity of milk fat as estimated by the tocopherol determination may force the reaction to deviate from its course, resulting again in oxidation of other substances than unstable lipids of the fat globules membrane. This particular phenomenon will be discussed in a following paper.

These observations also are in good agreement with the data of Beck *et al.*

(1) on the relation of carotene in milk fat to the development of oxidized flavors. These investigators have found a relationship between the color intensity of milk fat and the inhibition of oxidized flavors. However, Beck *et al.* have supplemented the rations with carotene concentrates during the barn feeding. Our analysis of some of the carotene concentrates by molecular distillation methods (11) revealed that their total tocopherol content was exceptionally high (approximately 20,000  $\mu\text{g.}$  per gram of concentrate).

The data we have presented are conclusive in showing that there is a relationship between the tocopherol and carotenoid contents of the milk fat as influenced by the roughages fed to the cow even though it might merely reflect parallel intakes of these two vitamins on the particular diet studied (13). It also has been shown that the promotion of oxidized flavors in milk products containing ascorbic acid, and which are associated with deterioration of milk fat, is apparently dependent on the stability of tocopherols and that the destruction of vitamin A and carotene follows that of tocopherols (9). Consequently, it would be logical to assume that the stabilizing effect on milk of carotene concentrate fed to the cows (1), largely was due to the increase in tocopherol content of the fat and not to that of carotene and that the latter is only a coincidental factor.

#### SUMMARY

The tocopherol, carotenoid and vitamin A content of cow's milk was determined for Holstein, Guernsey, Brown Swiss and Jersey cows during both pasture and barn feeding. Large variations were found in the tocopherol, carotenoid and vitamin A content of milk fat between individual cows of the same breed, between different breeds and between seasons.

As an average, the fat obtained from Guernsey milk was highest in tocopherol content with 3033  $\mu\text{g.}$  per 100 g. of fat, and Holsteins was lowest with 2220  $\mu\text{g.}$  Pasture milk contained more tocopherols than winter milk.

A significant positive correlation between the tocopherol and carotenoid content of milk was found but tocopherols and vitamin A were not correlated.

There is a relationship between the tocopherol content of the fat and the ability of milk to resist the oxidized flavors. A high proportion of samples of milk which contained less than 2500  $\mu\text{g.}$  of tocopherols per 100 g. of fat were unstable and developed oxidized flavors during the storage tests.

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# ISOLATION OF OVA FROM THE LIVING BOVINE<sup>1, 2</sup>

ARTHUR E. DRACY<sup>3</sup> AND W. E. PETERSEN

*Minnesota Agricultural Experiment Station, St. Paul*

Transfer of sperm cells by artificial means has greatly enhanced the use of the good proven sire. Transfer of the egg from good proven cows likewise would enhance the dissemination of good germ plasm to the extent that the method would be successful and multiple ovulation could be induced. The first step in attaining such an objective is the recovery of the fertilized egg from the cow.

The potentialities and problems of ovum transfer have been known for some time. The possibility of transferring fertilized ova from one individual to a foster mother has been adequately demonstrated in rats (2, 3, 4) and rabbits (5). Each of the above mentioned experiments required sacrificing the donor which in itself defeated much of the purpose of the experiment. However, Allen (1) has successfully isolated unfertilized monkey ova by a combination of surgery and flushing the oviducts. A similar procedure has been described by Umbaugh (6) as a means of securing ova from the cow. Surgery, although not extremely difficult on cattle, is not entirely satisfactory. Thus, a series of surgical and nonsurgical experiments have been undertaken to find a practical means of securing ova for transfers.

## EXPERIMENTAL PROCEDURES AND RESULTS

Study of the isolation of bovine ova falls into two parts. (a) To make the ovaries more accessible by translocation or transplantation and (b) to recover fertilized ova without surgical intervention.

Over a period of years a number of different techniques have been employed in attempts to obtain ova from developed ovarian follicles. The idea at first was that if the mature egg could be obtained, it could be fertilized *in vitro* before transfer to a recipient. However, this procedure recently has been shown not to be feasible. Since the ovary is located where it cannot be reached except by rather complicated surgery, the approach was to transplant or translocate the ovary so as to make it more readily accessible. The following surgical procedures were attempted: (a) transplantation into the neck, (b) subcutaneous translocation, (c) translocation into the vagina, and (d) resectioning of the uterine horn.

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<sup>1</sup> These data are taken from a thesis presented to the graduate school of the University of Minnesota by Arthur E. Dracy in partial fulfillment of the requirements for a Ph.D. degree.

<sup>2</sup> Published as paper no. 2607, scientific journal series of the Minnesota Agricultural Experiment Station.

<sup>3</sup> Present address: South Dakota State College of Agriculture, Department of Dairy Husbandry, Brookings.

*Transplantation of the ovary into the neck.* The first attempts to isolate ova from the living bovine were conducted by removal and transplantation of the ovary under the skin in the neck muscle of the animal. It was thought that eggs could be removed easily from developed follicles in such a preparation and subsequently fertilized before transplantation. None of these experiments was successful because the transplanted ovaries did not function. After several unsuccessful attempts, this method of trying to isolate living ova was discontinued.

*Subcutaneous translocation of the ovary.* Another attempt was made to place the ovary where it could be more easily observed. This was done by removing the broad ligament and ovary from the pelvic arch and placing it subcutaneously in the paralumbar fossa without interrupting its circulation. Such transplants did not ovulate, probably due to the reduced temperature in the new environment.

*Translocation of the ovary into the vagina.* Two attempts were made to translocate the ovary into the vagina. In one animal a laparotomy was performed and an incision was made in the anterior portion of the vagina, adjacent to the junction with the uterus. The ovary then was sutured into the vagina. On the second animal, however, the ovary was secured in the vagina without a laparotomy. This was accomplished by making an incision in the anterior portion of the vagina. The ovary then was moved into the vagina through this incision and sutured into place. From all physical appearances the animals withstood surgery very well. However, the ovaries did not remain translocated because of the violent vaginal contractions.

In the first animal the ovary slipped back into place indicating that the surgical technique was not adequate. In the second animal the translocated ovary was secured more substantially, but this ovary in turn pulled back into place, taking with it a fold of the vaginal wall which encased the ovary and grew together. This formed a pus-filled pocket about 3 in. in diameter around the ovary.

*Exteriorizing the resected uterine horn.* Another attempt by the use of surgery to isolate living ova was undertaken by resectioning the horn of the uterus and bringing the cut end to the exterior surface with the view that the ovum could be recovered by flushing the stump of the uterine horn after descent of the egg. In spite of precautions taken, salpingitis resulted.

*Non-surgical techniques.* After the previous surgical experiments, it became obvious that some other technique had to be used for the isolation of ova from the cow. From unpublished data, it also became apparent that the ovum must be fertilized and pass through the oviduct, since *in vitro* fertilization was unsuccessful. Two different approaches were made. First, a tube was inserted through the cervix up to the orifice of the oviduct and, second, the uterus was flushed by entrance through the cervix.

*Insertion of a rubber catheter up to the oviduct.* A rubber catheter was inserted through the cervix and butted against the oviduct with the view of capturing the descending ovum in the catheter, from which it might be flushed after removal from the uterus. The catheter was inserted on the third day following ovulation and left until the end of the fourth day. In the first animal the

cervix appeared to be large and little difficulty was encountered in passing the rubber catheter through the cervix into the uterine horn. However, in subsequent animals the cervix was smaller and a dilating apparatus had to be devised for opening the cervix. This was done by inserting a 0.25-in. stainless steel probe over which was placed a sleeve to act as a cannula. With the hand in the rectum holding the cervix, the probe and cannula were guided past the cervical folds. The probe was removed and the catheter extended through the cannula into the uterine horn. The cannula then was removed, leaving the catheter in the uterine horn.

Another difficulty experienced in this method was that as soon as a foreign body entered the uterus, violent uterine contractions occurred which did not stop until the tube was forced out of the uterus. To keep the catheter in the uterus, a stiff wire was inserted inside its posterior end at the anterior end of the cervix. The wire was bent in an "S" curve just inside the cervix to hold the catheter in place. This helped some; however, several of the animals were able to force the catheter out of the horn and into the body of the uterus.

In these experiments seven attempts to isolate fertilized ova were conducted on four cows. Even though some of the cows were unable to force the catheter out of the uterine horn, fertilized ova never were recovered. One of the animals conceived, further substantiating the fact that ova did by-pass the tube. In view of these apparently insurmountable difficulties, the experiments were discontinued.

*Flushing the uterus with a physiological solution.* The principle involved in this method was to force a warm (about 100° F.) physiological solution into the uterine horn and then to recover the fluid containing the ovum. Numerous laboratory experiments with isolated ova suspended in a physiological saline solution proved that ova have a greater specific gravity than the solution. The increased specific gravity allowed the ova to settle quickly to the bottom of a French separatory funnel. This procedure seemed to have some possibilities for the separation of ova from large quantities of fluid.

The equipment for flushing the uterus was the following: a 0.5-in. stainless steel probe 36 in. long for dilating the cervix and a fitted stainless steel cannula 24 in. long. These instruments sufficed for normal animals. Smaller animals, such as heifers, required proportionally smaller dimensions, usually not smaller than a 0.25-in. probe.

The flushing part of the apparatus consisted of a tire pump, a 1-l. aspiratory flask to hold the fluid and a 0.125-in. Koroseal tube. One end of the Koroseal tube was fastened into the stoppered aspiratory flask. The other end was heated by a Bunsen burner and drawn to a point sealing the end of the tube. Holes then were made in the sealed end of the tube by holding a heated dissecting probe against the Koroseal tubing in such a manner that when fluid was forced out of the tube there was a backward action.

Because 4 days elapsed from the time the animal was in heat before the ovum reached the uterus, the seventh day was arbitrarily selected as the best time for attempted recovery of the eggs. Thus, when the animal was in heat, she was



bred naturally or artificially, and then on the seventh day the removal of the eggs was attempted.

The technique of using the instruments mentioned in a preceding paragraph was simple if certain steps were adhered to rather closely. The steps in isolating ova by flushing with a physiological solution were as follows: (a) The arm was inserted into the rectum and all the fecal material was removed. Ovulation was determined by the presence of one or more corpora lutea on an ovary. After this the uterus was palpated for any abnormalities. (b) The probe and cannula were inserted through the cervix by grasping the cervix with the hand in the rectum and guiding the instruments past the cervical folds similar to the rectal methods of artificial insemination. After the probe was through the cervix, it was directed to either horn by maneuvering the uterus to either side. The probe was removed and the cannula was left in the horn. (c) The Koroseal tube was inserted into the cannula and passed through it. The Koroseal tube was directed from the end of the cannula to the tip of the uterus by the hand in the rectum. When the Koroseal tube was in place, pressure was applied to the flask. As the pressure increased in the flask, fluid was forced through the Koroseal tube into the uterine horn and returned through the cannula by gravity and the aid of the contracting uterus. A receptacle was held at the external end of the cannula and all of the returning fluid collected. (d) After 1 l. of physiological solution was pumped into the uterus, the Koroseal tubing was removed and the returning solution caught in the receptacle. (e) After the fluid had been taken to the laboratory, it was transferred into a series of 125-ml. French separatory funnels and allowed to stand 20 min. This usually allowed ample time for the egg to settle to the bottom of the separatory funnel. However, there were experiments in which the ovum adhered to the sides of the glass. This possibility was reduced by swirling the funnel and allowing the fluid to resettle. (f) After the required time had elapsed, a few milliliters of the mucus plus liquid were withdrawn from the bottom of the separatory funnel and observed at 23 magnifications under the dissecting microscope. Since ova are more than  $100\ \mu$  in diameter, identification was easy at this magnification. However, for further identification, the ovum was removed from the fluid by means of a fine capillary pipette, a hanging drop slide of it was prepared and it was observed under a high dry objective lens.

From observations using high-power magnification and from photomicrographs, the ovum appeared to be in the late blastula stage. Great care had to be taken not to confuse the ovum with tiny air cells that appeared in the liquid. By focusing up and down, air cells showed a reflection that was not observed when an ovum was under examination.

Table 1 shows the results obtained by flushing the uterus with a physiological solution. In this experiment, 12 cows were flushed 37 times. During these 37 trials, 41 ova were recovered. The table shows that cow 504 yielded ten ova at one time and cow 37E yielded two ova at one time, indicating superovulation. These two animals each were injected subcutaneously with 1,500 units of pregnant-mare serum to produce superovulation. At the time, 1,500 units of pregnant-mare serum induced the liberation of ova. However, about 2 mo. later

without a repeated injection, 37E liberated at least 20 ova at one ovulation which were recovered at one flushing.

## DISCUSSION

The data presented on the ovary transplantation and translocation experiments indicate the impracticability of such methods for isolating ova from the living cow. Possibly too few experiments were conducted to prove that recovery could not be accomplished by these techniques. With improved surgical technique it may be possible to transplant and translocate the ovary with satisfactory results. However, with the available material and the techniques applied at the time the experiments were conducted, the impracticability of this method of approach was apparent.

TABLE 1  
*Ova recovered by flushing uterus with a physiological solution*

No. of cow	Date of flushing	Ova recovered	No. of cow	Date of flushing	Ova recovered
E600	10-30-47	0	E598	2-18-48	0
	3- 3-48	0		2-23-48	0
	3-24-48	0		7-19-48	0
	4-28-48	0		8- 5-48	0
	7-21-48	0	479	2-21-48	0
E638	2-26-48	0		3-15-48	1
	5-31-48	0	504	10-14-48	0
	6- 7-48	0		11-11-48	1
813	3-24-48	0		12- 8-48	1
E608	9-15-47	0		1-17-48	10
	9-25-47	0		4- 1-49	1
	10-27-47	0	B10	1-29-49	1
	2-18-48	0	37E	2- 8-49	2
	3- 7-48	0		2-23-49	0
A53	8-16-47	1		4- 2-49	20
	4-12-48	0	34W	2-24-49	1
	6- 7-48	0		4- 2-49	0
E598	9-10-47	0	40E	4- 4-49	1
	10-16-47	1			

Attempts to recover ova in a catheter were unsuccessful. The fact that one cow became pregnant suggests that the ovum by-passed the catheter. The likelihood of capturing ova by this method seems remote. Also, as soon as the tube entered the uterus, violent uterine contractions occurred and continued until the tube was forced out. The contractions may have had a devastating effect upon the ovum coming down the oviduct into the tube. Even though one animal became pregnant, the possibility remained that some ova may fail to enter the uterine cavity. The possibility of obstructing the oviduct may be remote, yet it must not be overlooked as a cause of failure for recovery of ova.

From the data presented in table 1, regarding the flushing of the uterus with a physiological solution, eggs were recovered 12 times from 37 trials yielding a total of 41 ova. Of the 41 ova recovered, 32 were due to superovulation. In these experiments, four of the cows used never yielded an ovum in 14 attempts. In 23 attempts eight yielded ova 12 times. Thus, if the first four animals referred to were non-breeders, which could be possible because some of the

animals had been bred numerous times and because of repeated nonfertility were transferred from the college dairy herd to the experimental herd, the possibility exists that these animals could be sterile. The other eight animals were considered fertile, since fertilized ova were recovered. This being the case, ova were recovered 12 out of 23 trials, indicating that these cattle released ova for fertilization approximately 49.5 per cent of the time. Cow A53, which had yielded one ovum, later was sold from the herd as a sterile animal. This also was true of cow E598. When cow 479 yielded an ovum, she was returned to the college herd to produce a calf the following year. With improved techniques for obtaining and observing ova, possibly a larger percentage may be recovered. To further consider the number of ova that could in all probability be recovered, it should be kept in mind that according to data gathered from artificial insemination associations, 45-60 per cent of the cattle conceive on first service. If this is true and if the unfertilized ovum degenerates while traveling down the oviduct, the possibility of collecting nonfertilized ova is rare. Therefore, not more than 60 per cent of the recovery should be expected. However, there may be a few individual animals which would yield an ovum each time they were bred, the same as there are some cows that become pregnant on first breeding.

#### SUMMARY AND CONCLUSIONS

A series of experiments was conducted to determine the possibility of recovering ova from the living cow.

All surgical methods, such as transplantation of the ovary, resectioning the uterine horn and translocating the ovary subcutaneously, have yielded negative results.

The method of inserting a catheter into the uterus of a cow in order to recover fertilized ova has proved impractical.

Instruments and techniques for recovering fertilized bovine ova without injury to the donor's reproductive tract have been developed.

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# A COLORIMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF THE DEGREE OF LACTOSE HYDROLYSIS<sup>1</sup>

FRANK E. POTTER<sup>2</sup>

*Bureau of Dairy Industry, Washington, D. C.*

For the quantitative determination of a single sugar, numerous methods are available. However, most of these methods are not satisfactory when applied to a solution containing two or more sugars. In the hydrolysis of lactose three sugars, lactose, glucose and galactose, are involved. In certain methods of analysis, bacterial ferments or yeast enzymes are used to destroy one or more of the sugars in a mixture, but this is often time consuming.

In search of a method to follow the degree of acid hydrolysis of lactose, Ramsdell (6) used the following procedure. The sum of the two hexoses, glucose and galactose, was determined by Barford's modified reagent. Shaffer and Somogyi's procedure and their reagent no. 50 were used to measure the reducing power of the sugars before and after destruction of the glucose with bakers' yeast. From the results obtained, the quantities of glucose, galactose and lactose were calculated.

Another method which has been used to follow the hydrolysis of lactose is a modification (7) of the Willstaetter and Schudel procedure (8). This has been used for pure lactose in solution and in various dairy products.

The saccharimeter can be used to follow the hydrolysis of some sugars but with lactose it lacks sensitivity. It can be shown both experimentally and by calculation that for a 5 per cent solution of lactose an increase of less than 0.5 degree rotation occurs for every 10 per cent of lactose hydrolyzed.

Recently, Benham and Despaul (1) developed a quantitative colorimetric method for the determination of glucose. They measured the intensity of the blue color produced by the sugar in the presence of ammonium molybdate and potassium dihydrogen phosphate on heating. They also found the method suitable for the determination of glucose in the presence of moderate amounts of sucrose and recommended the procedure for the determination of other sugars. Later, Benham and Petzing (2) adapted this method to the quantitative measurement of maltose and mixtures of maltose and glucose.

It is this colorimetric method upon which the following study was conducted for the determination of the sugars obtained in the hydrolysis of lactose in milk products.

## EXPERIMENTAL PROCEDURE

The molybdenum blue method of Benham and Despaul (1) was followed, except for slight modifications. To several 25-ml. volumetric flasks, 5 ml. of 0.02M

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<sup>2</sup> Present address: Department of Dairy Husbandry, Agricultural and Mechanical College of Texas, College Station.

potassium dihydrogen phosphate and 10 ml. of 7.5 per cent ammonium molybdate were added. Samples, whether of pure sugars or of mixtures, were added in quantities containing between 1 and 10 mg. of sugar and the contents made up to volume with distilled water. The flasks were stoppered, inverted several times to mix the contents and the stoppers removed. The flasks were covered with individual tin foil caps to prevent contamination from condensing steam and heated in a preheated autoclave at 100° C. for exactly 30 min. The flasks were removed and cooled at once in ice water to stop the reaction. The color intensity was determined with a Klett-Summerson photoelectric colorimeter using a colored glass filter to give a wave length of 640 m $\mu$ .

Aqueous solutions of pure sugars did not require any preliminary purification prior to analysis. However, with milk it was necessary to obtain a clear serum for analysis. Precipitating agents, such as trichloroacetic acid, phosphotungstic acid and salts of heavy metals used for precipitating milk proteins in various chemical tests on milk products, interfered in the subsequent color production.

The method adopted for preparation of milk samples was as follows: To 50 g. of whole milk (20 g. of condensed skim milk) in a 100-ml. volumetric flask, 5 ml. of 1N H<sub>2</sub>SO<sub>4</sub> were added and made to volume with distilled water. The flask was stoppered, the contents mixed thoroughly and filtered through Whatman no. 2 filter paper. A 10-ml. aliquot of the filtrate was removed and placed in a 200-ml. volumetric flask. To this, 50 ml. of distilled water and five to six drops of phenolphthalein indicator were added. The contents then were neutralized to the phenolphthalein end point with 0.1N NaOH. The flasks were placed in a boiling water bath for 15 min. to coagulate the heat coagulable protein and cooled to room temperature in a cold water bath. To the contents of flasks five to six drops of methyl red were added and 0.1N H<sub>2</sub>SO<sub>4</sub> acid was used to adjust the reaction to the methyl red end point. The flasks were made to volume with distilled water, contents mixed and filtered. A 10-ml. aliquot of the filtrate was placed in the 25-ml. color development flasks and the analysis completed as for sugar solutions.

The intensity of the blue color produced in the molybdenum blue reaction varies with the individual type of sugar. Results of preliminary investigations on sugar solutions containing 1 and 8 mg. of glucose, galactose, lactose and a mixture of glucose and galactose in equal parts are presented in table 1. The data show that the color produced with the glucose and galactose mixture is more than twenty times as great as an equal quantity of lactose. The small amount

TABLE 1  
*Klett-Summerson readings at 640 m $\mu$  for known quantities of sugar*

	Scale readings	
	1.0 mg. sugar	8.0 mg. sugar
Lactose	< 5	20
Glucose	65	885
Galactose	140	too dark to read
Glucose and galactose (equal parts)	102	660

of color produced by the lactose can easily be corrected for by determining a blank value.

In order to determine unknown quantities of glucose and galactose, it was necessary to establish a standard curve using known quantities of these sugars. Three determinations were made on solutions containing from 0.5 to 8 mg. of glucose and galactose in equal parts. The average results of these determinations are presented in figure 1. This standard curve was used for calculating the re-

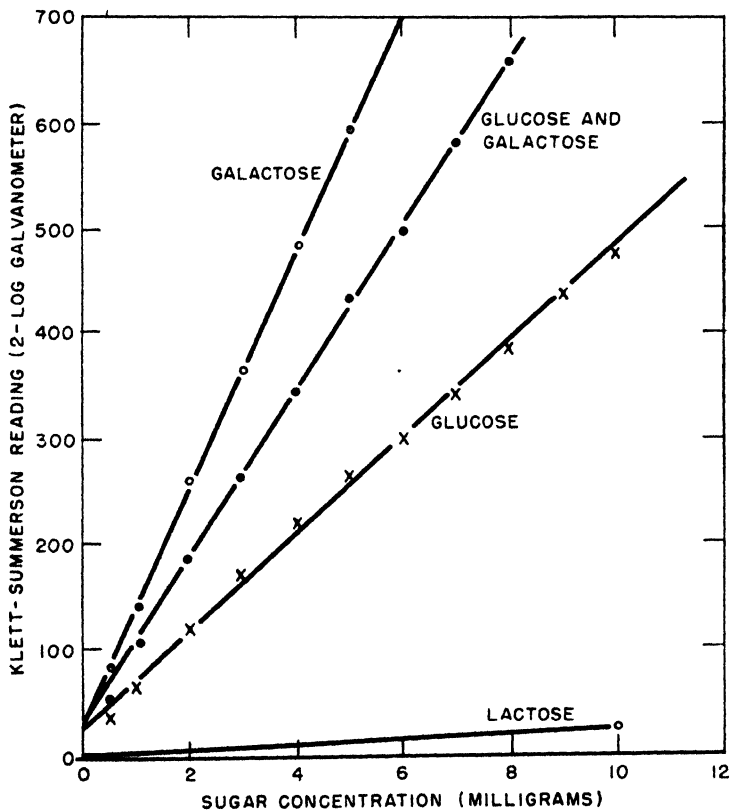


Fig. 1. Standard curves obtained from prepared solutions of known sugar content.

sults on unknown samples. The results of similar determinations on solutions of glucose, galactose and lactose also are presented in figure 1.

In analyzing an unknown sample of milk, the value obtained from the standard curve (mg. per aliquot) is converted to grams per 100 g. by a factor of 0.387 for whole milk. This factor is calculated by the method used by Hillig (3) for the quantitative determination of lactic acid.

To determine the accuracy of the molybdenum blue method when applied to milk, glucose and galactose in equal parts were added at the rate of 0.1 to 5.0 g. per 100 g. of whole milk. The results of seven trials in duplicate are presented in table 2.

TABLE 2

*Recovery of glucose and galactose added in equal parts to fresh whole milk*

Sample	Added	Recovered	Difference	Recovery
	(g./100 g.)	(g./100 g.)	(g./100 g.)	(%)
1a . . . . .	0.10	0.104	+ 0.004	104.00
b . . . . .	0.10	0.116	+ 0.016	116.00
2a . . . . .	1.00	0.956	- 0.044	95.60
b . . . . .	1.00	0.956	- 0.044	95.60
3a . . . . .	1.50	1.405	- 0.095	93.66
b . . . . .	1.50	1.405	- 0.095	93.66
4a . . . . .	2.00	1.989	- 0.011	99.45
b . . . . .	2.00	1.950	- 0.050	97.50
5a . . . . .	3.00	2.848	- 0.152	94.93
b . . . . .	3.00	2.980	- 0.020	99.33
6a . . . . .	4.00	3.870	- 0.130	96.75
b . . . . .	4.00	3.870	- 0.130	96.75
7a . . . . .	5.00	4.992	- 0.008	99.84
b . . . . .	5.00	4.938	- 0.062	98.76
Av. . . . .				98.70

These data indicate that the method possesses a high degree of accuracy and reliability. Recovery of the sugars was within 0.1 g. for all but three of the samples and the percentage recovery usually was within 5 per cent. The average recovery for the 14 analyses was 98.70 per cent.

In a supplemental series of tests involving the enzymatic hydrolysis of a lactose solution, comparisons were made between the Willstaetter and Schudel modified method and the method presented in this paper. Table 3 shows the results

TABLE 3

*The determination of glucose and galactose in a 5% solution of lactose at intervals during enzymatic hydrolysis*

Sample	Willstaetter & Schudel modification <sup>a</sup>	Molybdenum blue method	Difference
	(g./100 g.)	(g./100 g.)	(g./100 g.)
1	0.300	0.270	0.030
2	0.749	0.830	0.081
3	0.879	0.680	0.199
4	1.720	1.630	0.090
5	4.072	4.050	0.022

<sup>a</sup> These results obtained by G. Reed, Rohm and Haas Co., Philadelphia, Pa.

obtained by the two methods on five different samples. The quantity of glucose and galactose found by the two methods agreed within 0.20 g. per 100 g. for all samples and within 0.10 g. per 100 g. for all but one of the samples.

Since phosphomolybdic acid and ascorbic acid have been used for the determination of inorganic phosphate (4) and phosphomolybdic acid for determining ascorbic acid (5), experiments were conducted to determine the effect of ascorbic acid on this method of analysis. Fresh whole milk was divided into three lots and treated as follows: 1, control; 2 and 3, 50 and 100 mg. of ascorbic acid were added per liter of milk. Analysis of these samples (table 4) show that the addi-

TABLE 4

*Effect of the addition of ascorbic acid and the heating of milk on the normal blank values for milk*

Sample	Klett-Summerson reading	Glucose-galactose equivalent
Milk (control)	43	0.151
Milk + 50 mg. of ascorbic acid/l.	43	0.151
Milk + 100 mg. of ascorbic acid/l.	45	0.159
Milk (control)	45	0.159
Milk heated to 80° C. for 1 hr.	46	0.162
Milk heated to 80° C. for 2 hr.	46	0.162

tion of 50 mg. of ascorbic acid did not affect the normal blank value for milk. The addition of 100 mg. of ascorbic acid did increase the value slightly. However, since this is approximately five times the average amount of ascorbic acid found in milk, the normal variations in ascorbic acid would not have a significant effect on the blank values for milk.

Inasmuch as the heating of milk produces various reducing products, trials were conducted to determine the effect of heating. The results presented in table 4 show that the heating of milk at 80° C. for 1 or 2 hr. increased the blank value to a slight extent, but this increase was within the normal variation encountered for unheated milk.

In this study a blank value for milk has been found to vary from 0.150 to 0.170 g. per 100 g. expressed as glucose-galactose equivalent.

#### SUMMARY AND CONCLUSIONS

The colorimetric determination of sugars by the use of the molybdenum blue reaction as developed by Benham and Despaul (1) has been adapted to follow the enzymatic hydrolysis of lactose in solution and in milk.

The analysis of 14 samples of fresh whole milk containing from 0.1 to 5.0 g. of added glucose and galactose in equal parts per 100 g. of milk gave an average recovery of 98.70 per cent.

A blank value for milk was found to vary from 0.150 to 0.170 g. per 100 g. expressed as glucose-galactose equivalent. The addition of ascorbic acid or the heating of milk to 80° C. for 1 or 2 hr. did not have a significant effect on the blank values.

Values obtained by the colorimetric method for samples of hydrolyzed lactose agreed very closely with those obtained by a modification of the Willstaetter and Schudel method (7).

#### ACKNOWLEDGMENT

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# THE FURTHER DEVELOPMENT OF MILK REPLACEMENTS FOR DAIRY CALVES<sup>1, 2</sup>

J. B. WILLIAMS AND C. B. KNOTT

*The Pennsylvania Agricultural Experiment Station, State College, Pa.*

Previous work (15) has demonstrated that normal growth can be obtained in dairy calves by the use of limited amounts of saleable whole milk with a milk replacement. Numerous reports (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13) in the literature indicate the possibilities of raising dairy calves on limited amounts of whole milk and dry concentrates.

This report presents additional experiments relative to the improvement of formulas published previously. The principal objectives were to evaluate other plant and animal products and to develop a simpler formula. It was desired to study the comparative value of meat scrap, corn gluten meal, soybean oil meal, blood flour, dried skim milk and ground raw soybeans, nutri-soy and red dog flour in milk replacements. Previous work at this station (13) indicated a comparison was needed between dried brewers' yeast and distillers' dried solubles.

## EXPERIMENTAL PROCEDURE

The male Holstein calves used in the two trials were obtained from Pennsylvania state institutional herds. They were housed in individual solid-wall pens equipped with a water bowl, salt block, hay rack and a concentrate box. To prevent positional effects, the calves were placed at random throughout the artificially lighted and ventilated stable, maintained at a temperature of 65° F. by thermostatically controlled steam heat. Three measures of growth were taken each week, by the same person, at the same time and in the same order. The same person made daily observations on the condition of the feces of each calf. When a case of scours persisted for 24 hr., a 10-g. dose of sulfathalidine was administered orally followed by an additional 5-g. dose at each of the next two successive feedings.

*Trial 1.* Forty-eight calves were divided into eight comparable groups of six calves each on the basis of body weight, chest circumference and height at withers. Groups I through VII were placed on the experiment not later than the fourth day after birth and were fed the replacement formulas presented in table 1.

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<sup>2</sup> The data contained in this publication are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE 1  
Milk replacement formulas—Trial 1

Ingredient	Group						
	I	II	III	IV	V	VI	VII
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Dried skimmilk	50	20	20	10	10	20	5
Dried whey	10	20	20	20	20	20	10
Dist. dr. sol. (Corn)	10	20	20	20	20	20	20
Blood flour	10			10			5
Meat scrap		10	10	10	20	20	
Oat flour	5	10	10	10	10		20
Corn gluten meal		20		10	10		
Soybean oil meal			20	10	10	20	
Ground raw soybeans							40
Dextrose	7.75						
Brewers' dr. yeast	4.90						
Ground Fenugreek seed	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Irradiated yeast (9F)	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Stabilized vitamin A feed <sup>a</sup>	2.20	0.22	0.22	0.22	0.22	0.22	0.22
Minerals <sup>b</sup>	0.042	0.042	0.042	0.042	0.042	0.042	0.042
Dicalcium phosphate	2.5	2.5	2.5	2.5	1.0	1.0	2.5

<sup>a</sup> In mix no. 1 the vitamin A content of the supplement was 220,000 U.S.P. units/lb. In the other mixes the supplement contained 2,220,000 U.S.P. units/lb.

<sup>b</sup> Mineral mixture contained: Ferric citrate ( $\text{FeC}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$ ) 56.57%  
Cupric sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) 19.73%  
Manganese sulfate ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ) 21.59%  
Cobalt chloride ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ) 2.11%

They were fed the mixtures at 100° F. according to the following schedule: First through 4th day—dam's milk; 5th through 7th day—2.5 lb. whole milk, 0.25 lb. milk replacement, 2 lb. water (twice daily); 8th through 10th day—1.0 lb. whole milk, 0.5 lb. milk replacement, 4 lb. water (twice daily); 11th through 49th day—0.7 lb. milk replacement, 5 lb. water (twice daily); 50th to 56th day—0.7 lb. milk replacement, 5 lb. water (once daily).

Group VIII constituted the control group and was placed on the experiment not later than the fourth day after birth. They were fed a total of 372 lb. whole milk (3.4 per cent fat) excluding colostrum according to the following schedule: First day through 4th day—dam's milk; 5th through 14th day—8 lb. milk per day; 15th through 34th day—10 lb. milk per day; 35th day through 41st day—8 lb. milk per day; 42nd day through 49th day—4 lb. milk per day.

All groups of calves were fed a fair grade of timothy hay from birth to 8 wk. and good quality alfalfa from 8 wk. to end of 12 wk. trial, *ad libitum*. Calf starter was fed *ad libitum* until each calf was consuming the maximum of 6 lb. daily and then kept at that level of intake for the duration of the trial. The calf starter was prepared as follows: 406.5 lb. yellow corn meal, 300 lb. wheat bran, 400 lb. crushed oats, 140 lb. linseed oil meal, 280 lb. soybean oil meal, 140 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. dried skimmilk, 100 lb. dried corn distillers' solubles, 0.5 lb. irradiated yeast (9F), 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt and 3 lb. vitamin A feeding oil (2,724,000 USP units of A per pound).

*Trial 2.* Thirty-six calves were divided into six comparable groups of six calves each. They were fed the mixes in table 2 at a temperature of 100° F.

TABLE 2  
*Milk replacement formulas—Trial 2*

Ingredient	Group					
	I	II	III	IV	V	VI
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Dried skim milk	50	50	50	50	50	20
Dried whey	10	10	17	17	17	27.338
Dist. dr. sol. (Corn)	10	15	15	15	20	20
Blood flour	10	10	10			
Oat flour	5	5	5	5	5	
Soybean oil meal (exp. proc.)				10	5	
Nutri-Soy						15
Dextrose	7.75	7				
Red Dog flour						15
Brewers' dr. yeast	4.90					
Irradiated yeast (9F)	0.10	0.10	0.10	0.10	0.10	0.10
Stabilized vitamin A feed <sup>a</sup>	0.22	0.22	0.22	0.22	0.22	0.22
Minerals <sup>b</sup>	0.042	0.042	0.042	0.042	0.042	0.042

<sup>a</sup> The supplement contained 2,220,000 USP units/lb.

<sup>b</sup> Mineral mixtures same as table 1.

according to the following schedule: Birth through 7th day—colostrum and whole milk; 8th through 14th day—2 lb. whole milk, 0.2 lb. milk replacement, 2 lb. water (twice daily); 15th through 21st day—0.3 lb. milk replacement, 4 lb. water (twice daily); 22nd through 28th day—0.4 lb. milk replacement, 4 lb. water (twice daily); 29th through 42nd day—0.5 lb. milk replacement, 5 lb. water (twice daily); 43rd through 49th day—0.6 lb. milk replacement, 6 lb. water (twice daily); 50th through 56th day—0.6 lb. milk replacement, 6 lb. water (once daily). Since replacement I of trial 2 had been used in previous trials and the growth performance established, it was used as the control and the other mixes were deviations from it. Excellent quality second cutting mixed hay was fed *ad libitum* to 8 wk. and alfalfa from 8 wk. to determination of 12 wk. trial.

The number 1 and 4 calves in each group received the following concentrate in dry mash form: 416.5 lb. yellow corn meal, 300 lb. wheat bran, 400 lb. crimped whole oats, 100 lb. linseed oil meal, 300 lb. soybean oil meal (44 per cent), 150 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. dried skim milk, 100 lb. dried corn distillers' solubles, 0.5 lb. irradiated yeast (9F), 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt, 3 lb. vitamin A (2,270,000 USP units per pound in dry meal form). The number 2 and 5 calves in each group received the above concentrate in pellet form. The number 3 and 6 calves in each group received the following concentrate in pellet form: 390 lb. yellow corn meal, 100 lb. wheat bran, 100 lb. ground oats, 200 lb. linseed oil meal, 650 lb. soybean oil meal, 100 lb. alfalfa meal, 100 lb. fish meal, 300 lb. dried whey, 20 lb. ground limestone, 20 lb. steamed bone meal, 10 lb. iodized salt, 8 lb. feeding oil (1000 USP units of vitamin A and 400 USP units of vitamin D per gram), 1 lb. anise oil, 1 lb. irradiated yeast.

## EXPERIMENTAL RESULTS

*Trial 1.* Ration VII was lethal to all calves in the group, the calves succumbing at 27, 30, 31, 36, 43 and 58 days, respectively. The last calf was down in the stable for 7 days before being sacrificed for autopsy. Post-mortem revealed enlarged gall bladder, kidney discolorations, distended urinary bladder and excess fluid over the entire body. The condition in all the calves was characterized by muscular weakness and lack of coordination, although pain was not manifested. The calves maintained their appetites until death, although unable to stand up. One calf was lost from group I because of a hip injury, one calf from group IV was suspected actinomycosis, one calf from group V for cause unknown and one calf from group VI because of pneumonia.

All of the calves were easily taught to drink the warm replacement-water mixtures from open pails. Mix VII settled out quickly and mixes II, III, IV, V and VI settled out faster than was desirable. Mix I was very acceptable in water suspension. No serious or prolonged cases of scours occurred.

Growth data in table 3 indicates that calves in groups I and VIII made com-

TABLE 3  
*Mean daily gains in body weight, withers height and chest circumference, trial 1*

Group	Body wt.			Withers ht.			Chest circ.		
	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.
	(lb.)	(lb.)	(lb.)	(cm.)	(cm.)	(cm.)	(in.)	(in.)	(in.)
I	0.45	0.95	1.20	0.13	0.12	0.13	0.07	0.10	0.10
II	0.29	0.71	0.96	0.09	0.10	0.11	0.05	0.07	0.08
III	0.30	0.74	0.98	0.08	0.09	0.11	0.05	0.07	0.07
IV	0.29	0.52	0.79	0.09	0.10	0.10	0.01	0.04	0.06
V	0.26	0.68	0.95	0.11	0.11	0.11	0.01	0.06	0.08
VI	0.36	0.75	0.93	0.08	0.10	0.10	0.05	0.07	0.08
VII									
VIII	0.82	0.89	1.23	0.15	0.12	0.14	0.08	0.08	0.10

parable and uniform gains, except that group I calves made less gains the first 4 wk.; however, the appearance and well-being of these two groups of calves were superior to that of other groups. Also, the average consumption of calf starter was significantly less the first 8 wk. for groups I and VIII than for the other groups as presented in table 5.

From these growth data it would seem that corn gluten meal and soybean oil meal are comparable as sources of protein in conjunction with 20 per cent dried skimmilk powder. It also would appear that meat scrap alone is a better protein source than equal amounts of meat scrap and blood flour when dried skimmilk is used at the 10 per cent level.

*Trial 2.* As in trial 1, palatability was not a problem. All mixes remained in the warm water suspension without difficulty. There were no fatalities among any of the groups, although one calf in group VI failed to make satisfactory gains. The differences in daily gains (table 4) were not significant according to

TABLE 4

*Mean daily gains in body weight, withers height and chest circumference, trial 2*

Group	Body wt.			Withers ht.			Chest circ.		
	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.	4 wk.	8 wk.	12 wk.
	(lb.)	(lb.)	(lb.)	(cm.)	(cm.)	(cm.)	(in.)	(in.)	(in.)
I	0.48	1.00	1.24	0.12	0.14	0.14	0.05	0.08	0.09
II	0.46	0.98	1.32	0.11	0.13	0.14	0.05	0.07	0.09
III	0.21	0.89	1.10	0.10	0.13	0.14	0.03	0.07	0.08
IV	0.57	0.88	1.06	0.11	0.12	0.13	0.03	0.07	0.07
V	0.32	0.82	1.11	0.08	0.12	0.13	0.03	0.07	0.08
VI	0.39	0.79	0.98	0.09	0.11	0.11	0.00	0.06	0.08

the methods of Snedecor (14).

Table 5 presents the average consumption of calf starter. The difference in

TABLE 5

*Average consumption of calf starter at 8 and 12 wk.*

Group	First trial		Second trial	
	Av. consumption		Av. consumption	
	8 wk.	12 wk.	8 wk.	12 wk.
	(lb.)	(lb.)	(lb.)	(lb.)
I	45	171	71	190
II	60	187	71	206
III	58	185	60	171
IV	56	175	62	173
V	59	178	56	173
VI	60	184	59	160
VII				
VIII	47	161		

calf starter consumption up to 8 wk. of age between the group I calves in trial 1 and groups I and II in this trial probably was due to the differences in amount of the milk replacement fed. Each calf in trial 1 was fed 64 lb. of milk replacement and in trial two each calf received 41.2 lb. of milk replacement. Calves in groups I and II consumed a great deal more calf starter to 8 wk. and 12 wk. than did the calves in the other groups, and mean daily gains were higher, although not statistically significant. Further experimentation is planned in respect to the feeding of pellets *versus* mash, and the results of this trial will be reported when additional data are available.

The difference in daily gain between groups I and II and group III is difficult to explain. It may be that a combination of dried whey and dextrose is more beneficial to the infant calf than dried whey alone. Work is in progress at the present time on this phase. The growth data for groups I and II indicate distillers' dried solubles can effectively replace dried brewers' yeast. Growth was rather poor in the calves that received the Nutri-soy-red dog flour diet. Scouring was not a problem in any of the groups, although the group VI calves were rough-coated and generally unthrifty when compared to the calves in groups I and II.

## SUMMARY

Ground raw soybeans were not satisfactory when used at a 40 per cent level in the formula studied. All calves in a group of six died between the 29th and 58th day of age. Soybean oil meal and corn gluten meal were of equal value as a source of protein in these milk replacement formulas. Rations containing 50 per cent dried skimmilk gave consistently better results than those containing 20 per cent or less of this ingredient. Dried corn distillers' solubles effectively replaced dried brewers' yeast.

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# VANILLAS AS ANTIOXIDANTS IN POWDERED ICE CREAM MIXES

HARRY PYENSON<sup>1</sup> AND P. H. TRACY

*Department of Food Technology, University of Illinois, Urbana*

In a previous study, Pyenson and Tracy (1) has shown that a pure six-fold vanilla concentrate made from Bourbon and Mexican beans had antioxidant properties in powdered cream. Therefore, it was desirable to study other vanillas, both pure and artificial, and vanilla compounds to determine whether they also had antioxidant properties. This study was conducted with powdered ice cream mixes rather than with powdered cream, as vanillas usually are added to powdered ice cream mixes for flavoring. If certain vanillas do act as antioxidants, they then would serve a two-fold purpose in the powdered ice cream mixes. The results obtained on powdered cream mixes probably would be quite similar to those reported here on powdered ice cream mixes.

## EXPERIMENTAL PROCEDURE

A 1,100 lb. batch of liquid ice cream mix was made having the composition of 12 per cent butterfat, 11 per cent m.s.n.f., 15 per cent sugar (only one-third of the sugar was added before drying) and 0.2 per cent Dariloid. This mix was made from 35 per cent sweet cream, 34 per cent total solids condensed skimmilk and 9 per cent total solids skimmilk. The mix was pasteurized at 160° F. for 30 min., homogenized on a two-stage machine at 2,500 and 500 lb. pressure per in.<sup>2</sup>, then cooled to 40° F. and held over-night.

The liquid mix analyzed 13.39 per cent butterfat and 31.92 per cent total solids. Fifteen batches were dried on an experimental spray drier. The kinds and amounts of vanillas or vanilla products added as antioxidant are given in table 1. Each batch of powdered ice cream mix was divided into two parts, one

TABLE 1  
*Flavoring materials used in powdered ice cream mixes*

Batch no.	Amount	Kind of flavoring material	Brand
	(%)		
1	0		—
2	0.1	Conc. Bourbon and Mexican vanilla	A
3	0.1	Conc. Bourbon vanilla	A
4	0.3	Regular vanilla extract (Mexican)	A
5	0.3	Regular vanilla extract (Tahiti 100%)	A
6	0.1	Conc. pure vanilla extract	B
7	0.1	Conc. vanilla extract	C
8	0.2	Powdered pure vanilla	D
9	0.1	Powdered vanilla (Tahiti and Vanillin)	D
10	0.1	Conc. imitation vanilla	A
11	0.01	Methyl vanillin	A
12	0.01	Ethyl vanillin	A
13	0.0025	Vanillic acid	E
14	0.01	Coumarin	E

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<sup>1</sup> At present Director of Research, Reddi-Whip, Inc., 3938 Lindell Blvd., St. Louis 8, Mo.



part being air-packed and the other part nitrogen-packed. The letter *N* in the batch numbers indicate that the samples were nitrogen-packed.

In drying, the batches were preheated at a temperature of 145° F. and spray-dried at a pressure of 1,000 lb. per in.<sup>2</sup> using a number 69 nozzle and a 2-20 core. The inlet temperature was 310° F. and the outlet temperature was kept as close to 180 to 190° F. as possible. The powder was packed in no. 1 picnic cans, 150 g. of powder to each can. The cans were stored at a temperature of 75 ± 5° F., periodically analyzed for headspace oxygen by the method of Van Slyke and Sendroy (2) and judged for flavor by two or more judges after reconstituting with water in a Stevens mixer to original composition.

The moisture contents of the powdered ice cream mixes, as determined by the Mojonnier method, are recorded in table 2.

TABLE 2  
*Moisture content of powdered ice cream mixes*

Batch no.	Moisture	Batch no.	Moisture
	(%)		(%)
1	0.63	8	0.88
2	0.92	9	1.18
3	1.23	10	1.08
4	0.95	11	1.24
5	0.71	12	0.83
6	0.96	13	1.05
7	0.79	14	0.75

### RESULTS

This study was conducted for 1 yr. and the results are summarized in table 3. During this year, seven analyses for oxygen and flavor were made at approximately 1, 2, 3, 4, 6, 9 and 12 mo. of storage. The freshly reconstituted powdered ice cream mixes all were scored 41 on flavor based on the ice cream score card adopted by the American Dairy Science Association in 1941.<sup>2</sup> The flavor scores were determined solely on whether or not samples were oxidized. Some other flavor criticisms noted on the powdered ice cream mixes also will be mentioned. Amounts of vanilla or vanilla compounds used were the quantities thought needed for proper flavoring. Whether the results would have been altered by using greater or lesser amounts of the vanillas or vanilla products is not known.

Some of the other flavor criticisms noted were cooked, strong vanilla, strong artificial, alcoholic and weak. In batches 5 and 5N, 0.3 per cent of a 100 per cent Tahiti extract gave a strong vanilla flavor. Batches 1 and 1N had a cooked flavor. A strong artificial vanilla flavor was obtained in batches 9, 9N, 10 and 10N. An alcoholic flavor resulted when 0.01 per cent methyl vanillin was used in batches 11 and 11N. Vanillic acid in the amount of 0.0025 per cent produced a weak flavor. One hundredth per cent coumarin gave a strong flavor to the ice cream mixes in batches 14 and 14N. A weak flavor was noted with 0.1 per cent of a five-fold vanilla extract in batches 7 and 7N and 0.2 per cent powdered vanilla in batches 8 and 8N. Of all the vanillas or vanilla materials tested, the

<sup>2</sup> Excellent, 40 and above; good, 37.5-39.5; fair, 35.5-37.5; poor, 35.5 and below.

most pleasant flavor was produced by the powdered vanilla used in batches 8 and 8N. The harsh flavors produced by methyl vanillin, ethyl vanillin, vanillic acid and coumarin might be as objectionable or even more objectionable than oxidized flavor in a commercial product.

All of these vanillas and vanilla compounds were added to the ice cream mixes at the time of preheating just before spray-drying at a temperature of 145° F. The processing or the drying operations did not seem to affect the intensity of the vanilla flavor or vanilla compounds of the reconstituted powdered ice cream mixes.

Table 3 gives a resumé of the changes obtained in oxygen concentration in the headspace gas and the palatability of air-packed and gas-packed powdered ice cream mixes containing vanilla and vanilla compounds as antioxidants. Air-packed control batch no. 1 had a strong oxidized flavor at 37 days and the oxygen in the headspace gas already had started to diminish. After 1 yr. most of the oxygen had been used up and after about 6 mo. the flavor was so oxidized it was given a score of zero. The nitrogen-packed control (1N) also had become oxidized at 37 days storage but the oxidized flavor was not as strong as the air-packed samples throughout the storage period. The vanillas used were much more effective as antioxidants in nitrogen-packed samples than in air-packed. Most air-packed samples containing vanillas were oxidized after only a few months of storage. None of the nitrogen-packed samples containing vanillas were oxidized after 1 yr. of storage at room temperature.

Methyl vanillin, ethyl vanillin, vanillic acid and coumarin had antioxidant properties in powdered ice cream mixes. These compounds were almost as effective in air-packed as in nitrogen-packed samples. Samples containing methyl vanillin (11 and 11N) did not develop an oxidized flavor in either air-packed or nitrogen-packed samples held for 1 yr. Samples containing ethyl vanillin (12 and 12N) showed similar results except that at the sixth month storage period, the nitrogen-packed sample had a slightly oxidized flavor but was not criticized for oxidized flavor at the 9- or 12-mo. periods.

That the vanillas do not mask the oxidized flavors was shown in a previous paper (1). Nevertheless, this possible masking was checked again by adding one of the vanillas to the oxidized control sample. The results again indicated that there was little, if any, masking of the oxidized flavor by the vanilla flavors.

The gas analysis data in table 3 indicate that when vanilla or vanilla compounds were used, there was more oxygen left in the headspace gas than in the headspace gas of the control samples at the end of the storage period. This would indicate that less oxygen was used for oxidation in the powdered ice cream mixes and further proof that the vanillas and vanilla compounds tested have anti-oxidogenic properties.

#### DISCUSSION

All the products studied retarded or prevented the development of an oxidized flavor suggesting the presence of compounds capable of retarding oxygen uptake by the unsaturated fatty acids or phospholipids present in the powder.

TABLE 3

*Changes in oxygen concentration in headspace gas and palatability of air-packed and gas-packed powdered ice cream mixes containing flavoring materials*

Batch no.		Days of storage at room temperature						
		37	63	95	127	191	263	365
1	% Oxygen	18.46	19.47	18.78	17.28	12.49	3.66	2.48
	Flavor	35*	33*	30*	29*	0*	0*	0*
1N*	% Oxygen	2.09	2.52	2.10	2.08	1.05	0.00	0.00
	Flavor	37*	35*	33*	33*	32*	30*	25*
2	% Oxygen	20.00	19.33	18.68	18.08	16.53	13.15	13.52
	Flavor	38*	37.5*	37*	36*	34*	30*	25*
2N	% Oxygen	2.69	2.15	2.79	1.41	1.68	0.00	2.16
	Flavor	41.	40.5	40.	39.5	39.	39.	39.
3	% Oxygen	20.18	20.08	19.75	19.22	17.40	11.53	5.74
	Flavor	40.5	39*	38.5*	37.5*	36*	34*	32*
3N	% Oxygen	3.02	2.76	2.30	1.79	1.74	0.91	1.24
	Flavor	41.	40.5	40	39.5	39	39	39
4	% Oxygen	20.49	19.83	19.84	18.76	17.79	13.39	7.14
	Flavor	40.5	39*	39*	38*	37*	35*	33*
4N	% Oxygen	3.29	2.65	2.72	2.08	1.27	1.91	0.83
	Flavor	41.	40	40	39.5	39	39	39
5	% Oxygen	19.41	19.86	19.95	18.51	17.02	10.42	4.34
	Flavor	40.5	40	39.5	39	39	38	36*
5N	% Oxygen	3.24	3.61	3.07	2.50	1.95	1.01	0.89
	Flavor	41	40	40	39.5	39	39	39
6	% Oxygen	20.00	19.85	20.08	19.93	18.62	15.45	7.81
	Flavor	40.5	40	39.5	39	39	38	37*
6N	% Oxygen	3.45	4.01	3.87	2.91	2.83	2.58	1.55
	Flavor	41	40	40	39.5	39	39	39
7	% Oxygen	20.10	19.71	19.71	19.24	18.13	14.41	7.94
	Flavor	40.5	40	39.5	39*	38*	37*	35*
7N	% Oxygen	3.12	3.42	2.95	2.86	2.68	2.04	0.52
	Flavor	41.	40.	40.	39.5	39	39	39
8	% Oxygen	20.42	20.45	20.15	19.81	19.59	16.76	15.43
	Flavor	40.5	40.	39.5	39	38.5	38.5	37*
8N	% Oxygen	3.06	3.14	3.39	2.75	2.84	2.94	1.18
	Flavor	41.	40	40	39.5	39.5	39.5	39
9	% Oxygen	19.79	20.32	20.00	19.04	18.50	16.54	12.73
	Flavor	40.5	39.5*	39*	39*	38*	38*	37*
9N	% Oxygen	2.74	2.62	2.30	2.33	2.65	1.72	1.82
	Flavor	41	40	40	39.5	39	39	39
10	% Oxygen	20.78	20.17	19.90	20.09	19.25	17.52	15.80
	Flavor	40.5	40.	39.5	39	38.5*	38*	37*
10N	% Oxygen	3.02	2.91	2.74	2.28	1.88	1.76	1.36
	Flavor	41	40	40	39.5	39	39	39
11	% Oxygen	20.28	20.43	20.21	19.88	19.71	17.92	14.40
	Flavor	40.5	40.	39.5	39	38.5	38	38
11N	% Oxygen	3.77	2.93	2.53	3.17	2.28	2.09	1.13
	Flavor	41.	40	40	39.5	39.	38.5	38.5
12	% Oxygen	20.02	20.53	20.11	20.34	19.04	17.68	15.74
	Flavor	40.5	39.5	39.5	39	38.5	38	38
12N	% Oxygen	3.14	2.81	2.81	2.78	2.63	2.25	2.44
	Flavor	41.	40	40	39.5	38*	38.	38.5
13	% Oxygen	20.38	20.57	19.12	19.38	18.05	15.50	8.84
	Flavor	40.5	40.	39.5	39	38*	37*	36*
13N	% Oxygen	3.33	2.57	2.57	2.73	2.98	2.29	1.16
	Flavor	41	40	40	39.5	39	39	39
14	% Oxygen	20.15	20.09	20.11	18.88	18.10	12.14	8.48
	Flavor	40.5	40	39.5	39	38.5	38.	37*
14N	% Oxygen	2.98	2.75	2.67	2.39	1.82	1.35	0.89
	Flavor	41	40	40	39.5	39	39	39

\* N indicates samples were nitrogen-packed, others were air-packed.

\* Oxidized.

The explanation for this action is thought to be the structural formation of these compounds.

The structural formulas of methyl vanillin, ethyl vanillin, vanillic acid and coumarin are similar to certain compounds that are known to have antioxygenic properties. At low concentrations numerous phenolic substances have the ability to inhibit the autooxidation of fats. The most effective phenols are those which have some type of oxygen linkage in the ortho and para positions, or both, to the hydroxyl group. Some of the best known antioxidants of this type are hydroquinone, the tocopherols, gum guaiac and nordihydroguaiaretic acid.

Vanillin is the mono-methyl ether of protocatechuic aldehyde, the methoxy group being in the meta position to the aldehyde group. Vanillin is prepared commercially by synthetic methods from eugenole, which yields first iso-eugenole, or from the glucoside coniferin, which yields first coniferyl alcohol. When iso-eugenole and coniferyl alcohol are oxidized, vanillin is formed.

Vanillic acid is the mono-methyl ether of protocatechuic acid with the methoxy group in the meta position to the acid group. It is the acid corresponding to the aldehyde vanillin.

Ethyl vanillin has the same chemical structure as methyl vanillin, except that the ethoxy group is in the meta position instead of the methoxy group. Ethyl vanillin would be the mono-ethyl ether of protocatechuic aldehyde (4 hydroxy 3-ethoxy benzaldehyde).

Coumarin is an odoriferous compound present in tonka beans, the extract of which is used as a substitute for vanilla in some imitation vanilla extracts.

The addition of flavoring compounds having the ability to prevent oxygen uptake by the fatty materials should prove to be a very convenient method of extending the shelf life of a number of foods. It is possible that the extent to which vanilla flavors have been helpful in this respect has not been fully appreciated.

#### SUMMARY

Studies were made of nine vanillas and four vanilla compounds in powdered ice cream mixes held for 1 yr. at room temperature. The products used represented five different manufacturers of vanillas or vanilla compounds.

Changes in the oxygen concentration of the headspace gas and palatability studies indicated that these vanillas and vanilla compounds have antioxygenic properties in powdered ice cream mixes. The addition of these vanillas or possibly the vanilla compounds would serve a two-fold purpose in powdered ice cream mixes, i.e., as flavoring and as an antioxidant.

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# PASTEURIZATION EFFICIENCY OF THE VACREATOR WHEN USED ON ICE CREAM MIX

P. H. TRACY AND RICHARD PEDRICK<sup>1</sup>

*Department of Food Technology, University of Illinois, Urbana*

AND

H. C. LINGLE

*Research Laboratories, Cherry-Burrell Corporation*

The Vacreator<sup>2</sup> is a continuous flow type of high-temperature, short-time pasteurizer so constructed as to include three successive stages, each of which operates under a pressure lower than that of the atmosphere (fig. 1). The rapid heating is accomplished by the gravity fall or rain of the liquid through a chamber of expanded steam. This method of heating with steam is the reverse of steam injection. Rapid cooling results from the evaporation of moisture which occurs when the liquid passes to the lower pressure areas existing in the successive stages. A water actuated ejector-type condenser is an integral component of the machine and its high velocity water jet serves to maintain vacua, condense vapors and entrain and eject non-condensable gases.

In normal operation, the incoming and outgoing temperatures of the milk product being processed are maintained at practically the same levels so that the moisture content of the product leaving the Vacreator is essentially that of the product entering the machine. The novel features of the process are the rapid heating of the fluid particles resulting from the controlled addition of more steam than is required to heat the product to a pasteurizing temperature followed by its removal in the second and third chambers, thus providing steam distillation. Temperature control is effected by regulating the pressures maintained within the chambers and not by changing the amount of steam being used. The process should provide an excellent means not only of operating continuously, but also of complete pasteurization without injury to flavor. There also is the possibility of actually improving flavor through the removal of undesirable volatile substances present in the milk product.

The merits of the Vacreator process as applied to cream for buttermaking, milk for cheese making and ice cream mix have been studied extensively by Wilster. In reviewing his own work as well as that of others, Wilster (1) reports that the main advantages of the process are improvement of flavor and high efficiency of pasteurization.

In operating the Vacreator, temperatures can be varied. The usual range, however, is 195 to 205° F. in the first chamber, 160 to 180° F. in the second chamber and 110° F. in the third chamber, with vacuum readings in the three chambers varying from 4 to 9 in. in the first, 15 to 25 in. in the second and 28 in. in the third.

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<sup>1</sup> Now associated with the Dean Milk Co., Research Laboratories, Rockford, Illinois.

<sup>2</sup> Vacreator—a trademark for vacuum pasteurizers. Registered U. S. Pat. Off. and Canada.

High-temperature, short-time methods of pasteurization for ice cream mix are not commonly used in this country, as public health officials have not as yet established standards for the various time and temperature combinations possible. Commercial and public health interest in the use of the Vacreator for mix manufacture led to the study reported herein.

# EXPERIMENTAL PROCEDURE

A no. 3 size Vacreator, having a rated maximum capacity of 3,000 lb. of product per hour, was used (fig. 1). A positive type variable speed stainless steel pump was provided to deliver the product to the Vacreator. A standard two-

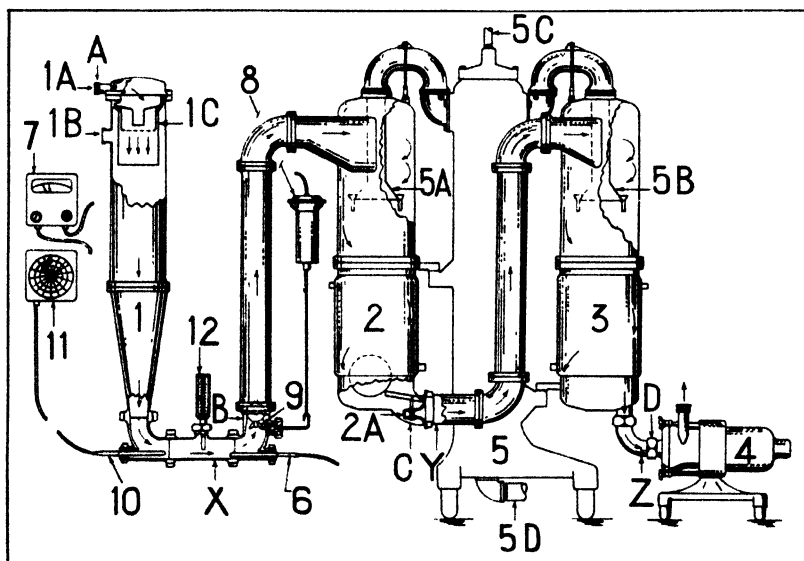


FIG. 1. Diagram of the Vacreator

- |        |  |      |   |
|--------|--|------|---|
| 1.     | First chamber  | 8.   | Regulator between Pasteurizing Temperature Controller and Equilibrium Valve |
| 1A.    | Product Inlet  | 9.   | Equilibrium Valve   |
| 1B.    | Steam Inlet  | 10.  | Bulb of Safety Thermal Limit Recorder                                       |
| 1C.    | Spray Pan  | 11.  | Safety Thermal Limit Recorder   |
| 2.     | Second Chamber   | 12.  | Pasteurizing Temperature Mercury Indicating Thermometer                     |
| 2A.    | Float Valve  |      |   |
| 3.     | Third Chamber  |      |   |
| 4.     | Product Discharge Pump                                       |      |   |
| 5.     | Ejector Condenser  |      |   |
| 5A-5B. | Vapor Intake Pipes to Ejector Condenser                      |      |   |
| 5C.    | Condenser Water Inlet  |      |   |
| 5D.    | Condenser Water Outlet Piped to Drain or Water Cooling Tower |      |   |
| 6.     | Bulb of Pasteurizing Temperature Controller                  |      |   |
| 7.     | Pasteurizing Temperature Controller                          |      |   |
|        |  | A-B. | First Chamber Effect  |
|        |  | B-C. | Second Chamber Effect   |
|        |  | C-D. | Third Chamber Effect  |
|        |  | X —  | Sampling Cock   |
|        |  | Y —  | Sampling Cock   |
|        |  | Z —  | Sampling Cock   |

stage centrifugal stainless steel pump discharged the product from the third chamber.

The Vacreator was equipped with vacuum and pressure gauges, indicating and recording thermometers and automatic steam control, as well as an automatic pasteurizing temperature controller.

Determination of time required for a liquid to pass through the Vacreator was made using an electric clock calibrated in hundredths of a second. Brine, flowing behind the clear water, upon contacting the first set of electrodes started the clock and stopped it when contact was made with the second set of electrodes.

With pump speeds and steam pressure constant, the rate of mix flowing through the Vacreator will remain constant. However, temperature in the first two effects, where bacterial destruction takes place, can be varied. It was desired to determine the significance of the temperature at these two points in the process, particularly in the first effect. Temperatures in the first effect were varied from 180 to 200° F. The temperature in the second effect was kept at 140° F. by removing the second chamber float valve or at 170° F. with the valve in place.

A mix containing 12 per cent butterfat, 11 per cent milk solids-not-fat and 15 per cent cane sugar was used, unless otherwise specified. The pasteurized mixes were inoculated with 24-hr. cultures of *Micrococcus freudenreichii* M25 just before vacreation took place. The cultures were prepared by growing on tryptone-glucose extract agar at 37° C. The growth was washed from the agar with sterile one-fourth strength Ringer's solution and the washings added to the mix. Samples of the vacreated mix were plated on tryptone-glucose-extract agar and incubated at 37° C. for 48 hr. before counting.

Samples were taken from the first chamber effect by two methods. One method was by gravitational fall into a sterile tube immersed in ice water connected to a cock on the first chamber. The second method consisted of drawing a sample into a continuously evacuated sterile flask. The latter method finally was adopted, as it gave instantaneous cooling of the sample.

As a control measure, a sample of each experimental mix was laboratory pasteurized at 155° F. for 30 min. in a sealed, sterile glass tube.

Experiments also were made to determine to what extent deviations from the normal procedure of operation would affect the pasteurization efficiency of the Vacreator.

#### RESULTS

As it would be difficult to determine at what point in its passage through the first chamber a mix particle reached the peak temperature, it was decided to measure the time required for a liquid to pass from the intake of the first chamber to the discharge of this chamber. Thus, the data obtained showed the length of time involved in heating to and holding at the indicated temperature of the first chamber. To accomplish this, electrodes were placed under the spray pan where the product first is exposed to live steam. A lead-covered cable was run from the clock through the steam piping into the first chamber to these electrodes. The

stop electrodes were placed immediately in front of the equilibrium valve, which is located at the discharge end of the first chamber. Salt solution was injected 6 in. upstream from the spray pan by means of a syringe. To obtain accurate readings, the resistance between the clock and each electrode was increased to the maximum that would still permit the clock to operate. This insured that only the peak concentration of salt would be timed as it passed each electrode.

This procedure was necessary due to the fact that the temperature of the solution used in these tests increased 80° F. between the two timing electrodes. Tests showed that the conductivity of the salt solution was higher at 190 than at 110° F. Because of this effect of temperature upon conductivity, the most accurate results were obtained by the method described of timing the peak concentrations of salt. Once set, the resistances to the electrodes were not varied, so the effect of varying the steam and product supply could be accurately determined.

When the steam supply used was reduced from 530 lb. per hour to 440, 320 and 230 lb. per hour (table 1), the average time of exposure to the temperature

TABLE 1

*Time (seconds) required for water to pass through first chamber of Vacreator*

A. Variable steam supply.

Steam line pressure* (psi)	38	28	18	10
Steam supply (lb./hr.)	530	440	320	230
	0.69	0.78	0.75	0.85
	0.82	0.78	0.75	0.90
	0.75	0.79	0.88	0.94
	0.76	0.78	0.82	0.84
	0.79	0.73	0.81	0.97
	0.77	0.73	0.80	0.90
	0.78	0.83	0.72	0.90
	0.85	0.84	0.84	0.86
	0.69		0.86	0.86
Av.	0.75	0.78	0.80	0.89

B. Variable operating capacity.

Vacreator capacity (lb./hr.)	Steam line pressure (38 psi)* Steam supply (530 lb./hr.)			
	1800	2600	3800	5300
	0.75	0.72	0.70	0.62
	0.64	0.69	0.57	0.61
	0.68	0.72	0.64	0.59
	0.79	0.64	0.61	0.61
	0.80	0.64	0.68	0.63
	0.81		0.69	
	0.71		0.67	
	0.74		0.61	
Av.	0.74	0.68	0.64	0.61

Infeed temperature 110° F.; first chamber temperature 190° F.; second chamber temperature 170° F.; quantity of salt used 115 ml.; capacity of machine 1800 lb./hr.  
\* As delivered through a 0.5 in. diameter-fixed orifice.

of the first chamber was increased from 0.75 sec. to 0.78, 0.80 and 0.89 sec., respectively. When the capacity was increased from 1,800 lb. per hour to 2,600, 3,800



and 5,300 lb. per hour, the time of exposure to the temperature of the first effect was reduced from an average of 0.74 sec. to an average of 0.68, 0.64 and 0.61 seconds, respectively.

Tests also were performed for the purpose of determining the length of time required for complete travel through the Vacreator. In running these tests, the second chamber float valve was removed. This was done in order to determine whether or not, when bacterial destruction effects of the first chamber were measured, the length of time in the second chamber would be sufficient to cause any additional bacterial destruction.

In these tests the stop electrodes were placed in a tee fitting at the discharge elbow leading from the third chamber. Pump capacities of 1,800 and 2,600 lb. per hour and steam pressures of 20 and 40 lb. were used (table 2).

TABLE 2

*Minimum time (seconds) required for complete passage through the Vacreator with second chamber float valve removed*

Capacity (lb./hr.)	2600	1800	1800
Steam line pressure <sup>a</sup> (psi)	40	40	20
Steam supply (lb./hr.)	550	550	320
	5.69	7.17	7.0
	5.68	6.85	6.9
	5.83	7.07	6.95
	5.33	8.03	7.01
	5.64	6.68	7.07
Av.	5.63	7.16	6.98

<sup>a</sup> As delivered through a 0.5 in. diameter-fixed orifice.

An increase in capacity from 1,800 to 2,600 lb. per hour reduced the time required for complete travel through the Vacreator from an average of 7.16 to 5.63 sec., whereas variation in the steam supply did not significantly alter the elapsed over-all time.

From these results it is evident that when the Vacreator is operated with the second chamber valve removed and the second chamber temperature is held at 140° F. for not more than 5 to 6 sec., a sample taken from the third chamber outlet will reflect only the lethal effect of the first chamber heat treatment. This makes possible the assumption that in tests performed for the purpose of determining the effect of the first chamber heat treatment, samples taken at the discharge end of the Vacreator will be as satisfactory as those taken directly from the first chamber.

A batch of mix after inoculation with *M. freudenreichii* M25 was preheated to 110° F. and passed through the Vacreator, using temperatures in the first chamber varying from 200 to 180° F. at 5 degree increments. The second chamber temperature was kept constant at 170° F. The mix discharge temperature was 110° F. The machine was operated at a capacity of 2,500 lb. per hour. Samples from the second and third chambers were taken by the vacuum method.

The results (table 3) indicate that the lethal effect of the second chamber when operated at 170° F. was not significant until the first chamber temperature

TABLE 3

*Relation of first and second chamber temperatures to bacterial destruction in mix*

Bacterial counts per ml. mix.							
First chamber temperature	Natural flora mix	After addition of culture	After first chamber treatment		After second chamber treatment of 170° F.	After third chamber treatment	Lab. past. count 155° F., 30 min.
			Gravity Sample	Vacuum Sample			
(° F.)							
200	6,000	760,000	250	500	170	2,900	2,800
200	15,500	630,000	470	300	800	990	1,600
195	20,000	2,200,000	1,100	870	1,570	1,200	3,500
195	800	4,200,000	1,800	3,200	1,200	850	3,900
190	1,700	1,500,000	750	6,200	460	1,070	1,100
190	12,000	3,200,000	1,250	5,200	580	720	2,800
185	19,000	4,300,000	18,000	110,000	720	1,200	2,700
185	1,200	3,600,000	22,000	90,000	1,200	1,500	3,200
180	20,000	4,300,000	127,000	320,000	37,000	20,000	4,000
180	20,000	5,200,000	290,000	530,000	22,000	9,000	5,200

dropped to 190° F. or below. Not until the first chamber temperature was reduced to 180° F. did laboratory pasteurization give results superior to those obtained by vacreation.

To better check the lethal effect of the first chamber, trials were run in which the second chamber float valve was removed and the temperature in this chamber maintained at 140° F. and below. This is not a normal procedure, but it made possible the elimination of any bacterial destruction in the second chamber. The first chamber temperature was maintained at 200° F., then dropped at 5° intervals to 180° F. The mix used first was pasteurized and then inoculated with *freudenreichii*. Samples were taken during each first-chamber temperature condition after the mix had traversed the entire vacreation process. As the second chamber retention time had been found to be approximately 5 sec. with the float valve removed, the time and temperature of this chamber had a negligible effect upon the destruction of the organisms. The removal of the second-chamber float valve had no effect upon the time and temperature maintained in the first chamber. The third chamber at 110° F. had no lethal effect.

This method provided an accurate measurement of the efficiency of the first chamber on which the automatic controls are mounted and in which most of the microorganism destruction takes place. The holding time in the first chamber was exactly that as normally maintained and the error encountered in drawing the sample directly from the first chamber, due to added holding time at a high temperature, was avoided. Sufficient time was allowed between sampling to permit the mix pasteurized at the preceding higher temperature to be completely removed from the system, thus avoiding possible contamination at the subsequent lower temperature pasteurization treatments. The preheating temperatures were standardized at 110° F., and the pump capacity was set at 2,500 lb. per hour in each trial. Results are presented in table 4.

If it is assumed that the heating done in the second chamber is to be considered only as a safety measure and that complete pasteurization must take place

TABLE 4  
*Pasteurization efficiency of Vacreator with second chamber float valve removed*  
 (Samples taken at the end of the process)

Trial	Natural flora of mix	After addition of culture	Bacteria counts per ml. of mix								Lab. past. samples 155° F. 30 min.
			First chamber temperatures								
			(Second chamber temperature 140° F.)								
			200° F.	195° F.	193° F.	191° F.	190° F.	189° F.	185° F.	180° F.	
1.	700	1,020,000	1,710	760	.	..	7,200		219,000	268,000	36,000
2.	6,300	2,200,000	2,600	2,500	.	..	11,000		32,000	240,000	7,000
3.	32,000	1,910,000	270	410	.	..	7,000		65,000	169,000	30,000
4.	27,000	1,040,000	190	570	.	..	23,700		184,000	249,000	1,850
5.	87,000	720,000	320	17,000	.	..	112,000		170,000	210,000	2,400
6.	120,000	2,200,000	180	370	.	..	16,000		190,000	270,000	2,750
7.	21,000	3,200,000		21,000	34,000	101,000		137,000			3,250
8.	17,000	2,300,000		820	2,600	90,000		89,000			3,250
9.	700	920,000		500	700	3,800		50,000			1,350

Note: The second chamber was maintained throughout these trials at the temperature of 140° F. or below, in order that the bacterial destruction of the first chamber alone could be measured.

TABLE 5  
*Pasteurization efficiency of Vacreator with second chamber float valve removed*  
*(Samples taken at the first chamber and at the third chamber outlet)*

Trial	Natural flora of mix	After addition of culture to mix	Bacteria counts per ml. of mix												Lab. past. samples 155° F. 30 min.					
			First chamber temperatures																	
			200° F.			198° F.			196° F.			194° F.				192° F.			190° F.	
			First effect	End of process		First effect	End of process		First effect	End of process		First effect	End of process			First effect	End of process		First effect	End of process
1	300	1,020,000	600	400	630	1,400	740	930	1,250	1,190	7,800	4,900				11,800	1,280			
2	270	820,000	500	660	530	690	580	620	760	7,700	1,150	6,200				13,400	2,100			
3	80	1,520,000	660	1,020	400	990	750	1,000	7,200	11,900	12,000	13,800				80,000	1,600			
4	530	810,000	420	430	580	650	570	620	760	4,000	5,000	21,800				53,000	3,600			
5	570	850,000	280	610	350	530	760	710	1,430	2,900	760	880				56,000	4,600			
6	530	720,000	280	290	310	460	510	610	890	930	4,400	6,200				52,000	16,100			
7	270	870,000	260	360	370	390	380	440	630	710	8,900	4,700				144,000	6,200			
8	690	840,000	340	390	240	350	350	260	360	530	7,700	2,300				16,900	11,100			

Note: The second chamber was maintained throughout these trials at the temperature of 140° F. or below, in order that the bacterial destruction of the first chamber alone could be measured.

by the time the mix enters the second chamber, then there is no question but that the first effect temperature must not drop below 190° F. It also is evident that the temperatures as high as 200° F. in the first effect are not necessary to obtain suitable bacterial destruction.

The study was continued using a mix that had been rendered nearly sterile before the addition of the culture. This was accomplished by circulating the mix through the Vacreator at 205° F. It was impossible to obtain a sterile mix due to the presence of Gram-positive, aerobic, spore-forming, rod-shaped organisms. First chamber temperatures were varied from 200 to 190° F. with 2° intervals. Samples were taken both from the first chamber and at the end of the process. The results (table 5) indicate, as in the previous experiment, that temperatures as high as 200° F. are not necessary for proper pasteurization. With one exception, (trial 3) results obtained at 194° F. were superior to those obtained in laboratory pasteurized samples. In general, results obtained at 192° F. were inferior to those obtained on the laboratory pasteurized samples.

Before accepting a new method or process for pasteurizing a liquid dairy product, it is necessary to know what safety precautions must be taken to prevent improper pasteurization from occurring due to accidental or willful misoperation of the process. Better bacterial results were obtained when the mix was passed through the Vacreator at temperatures of 194° F. or higher than when the mix was laboratory pasteurized at 155° F. for 30 min. (tables 3-5). However, the question might be raised as to what would be the result of (a) a reduced infeed temperature, (b) an increase in the infeed pump speed, (c) a sudden reduction in steam line pressures and (d) clogging of the spray pan in the first chamber. Accordingly, experiments using milk were conducted for the purpose of obtaining answers to these questions. Milk was selected as the test liquid for economy reasons and because preliminary studies had shown its suitability for such studies.

Two runs were made at a pasteurizing temperature of 190° F. combined with the abnormally low preheat temperatures of 70 and 49° F. (table 6). Operation at the 70° F. preheating temperature was satisfactory, and bacterial kills were excellent. This was in spite of the fact that the thermometer bulb which operates

TABLE 6

*Bacteria counts obtained with low product infeed temperatures, using whole milk*

Trial	No. 1	No. 2
Infeed temp. . . . .	70° F.	49° F.
Controller past. temp. . . . .	190°	190°
Actual past. temp. . . . .	186°-190°	182°-196°
Inoculated count . . . . .	12,400,000	3,500,000
1st chamber count/ml. . . . .	450	2,200
2nd chamber count/ml. . . . .	250	540
3rd chamber count/ml. . . . .	440	450

1st chamber samples were drawn into a continuously evacuated flask. In other tests, nine samples of the inoculated milk pasteurized in the laboratory at 148° F. for 30 min. had counts ranging from 88,000 to 266,000/ml.

The second chamber temperature was 170° F. The capacity was 2,640 lb./hr. and the steam supply was 530 lb./hr.

the recording thermometer and which is located at the entrance of the crossover tube showed fluctuations to as low as 186° F. This indicates that the product was not completely raised to 190° F. until almost out of the crossover tube.

The run at the 49° F. preheat temperature showed 14° F. fluctuations of the pasteurizing temperature, as well as an erratic discharge combined with vacuum fluctuations. A 0.5-in. orifice in the steam line limited the pounds of steam available to 550, and this amount of steam was not sufficient to give a uniform discharge temperature of 190° F. The product fluctuated to as low as 182° F. The safety thermal limit pump stop was not used, as it would have shut off the product flow. Even with these temperature fluctuations, the bacterial destruction remained equivalent to normal operation.

Under some operating conditions, such as a run lasting many hours, precipitated milk proteins or some other solid material might partially clog the holes of the spray pan. Under such conditions, the milk product would not fall as droplets through the live steam as in normal operation, but would run in a stream down the wall of the pasteurizing chamber. This conceivably could reduce the heat transfer and prevent all the product from being raised to a temperature that would give adequate pasteurization.

To study this possibility, 30 gal. of skimmilk were circulated through the Vacreator for 6.5 hr. The steam was superheated 10° F., and for parts of this run a preheat temperature of 125° F. was used so as to condense the product. At the end of the run, the amount of accumulated milk solids or burn-on was not excessive. All the burn-on was around the edge of the pan and not in the zone of the spray holes.

Since other products might cause a heavier burn-on and thus clog the spray pan, a pasteurizing test was made with the funnel to the spray pan closed with cork. The bottom of the pasteurizing chamber was temporarily removed to inspect the flow. Much of the product was seen to run down and drop off the spray guard, while some of it ran down the side of the chamber. No impairment of pasteurization efficiency resulted.

It also was realized that there might be a danger of inadequate pasteurization if the spray pan was accidentally left out when assembling the Vacreator. To exaggerate the conditions of flow that would then occur, a cork with a 0.75-in. diameter hole was placed in the funnel to the spray pan. With the spray pan removed, the product then fell in a solid stream, yet pasteurization again was satisfactory.

Tests were made to determine the pasteurizing efficiency of the Vacreator when operated above the rated maximum capacity of 3,000 lb. per hour. Runs were made at rates of 3,200, 3,800 and 5,300 lb. per hour. All samples taken, with the possible exception of one count (8,360 per ml.) at the highest capacity, indicated satisfactory bacterial reduction. The tests were made at a pasteurizing temperature of 190° F. (table 7).

The infeed pump was overloaded above the 3,800 lb. rate and, thus, could only be run for short periods without being stopped by the overload cut-outs.

TABLE 7

*Effect of overloading Vacreator upon bacterial destruction in whole milk*

Run no.	1	2	3
Capacity (lb./hr.) . . . . .	3,200	3,800	5,300
Inoculated count/ml. . . . .	750,000	2,100,000	3,300,000
First chamber count/ml.* . . . .	560	370	600
Second chamber count/ml. . . . .	420	300	8,360
Third chamber count/ml. . . . .	1,080	1,260	770

\* First-chamber samples were drawn into a continuously evacuated flask. In all 3 runs, the infeed temperature was 190°, the second chamber temperature was 170° and the steam supply was 530 lb./hr.

Vacreator operation at these excessive capacities was satisfactory but was attempted only for short periods. It is reasonable to assume that the operator would correct a highly overloaded situation shortly after it occurred in order to restore proper functioning of the Vacreator. Overloading as much as 50 per cent above the rated maximum capacity apparently did not result in unsatisfactory pasteurization of the milk.

A comparison was made of the results obtained with reduced, normal and excessive steam supply (table 8). In one test the steam line pressure was reduced

TABLE 8

*Effect of varying steam quantities on bacterial destruction*

Steam supply (lb./hr.)	530	320	230	800
Steam line pressure (psi.)	40	20	10	45
1st chamber temp. recorded .	190°	190°	182-185°	212°
2nd chamber temp.	170°	170°	170°	165°
Capacity (lb./hr.)	1,800	1,800	2,000	2,650
Inoculated count/ml.	4,800,000	2,730,000	1,470,000	390,000
1st chamber count/ml. .	1,210	880	700	100
2nd chamber count/ml.	930	320	500	
3rd chamber count/ml.	1,600	290	1,250	1,600

Steam line orifice removed. The infeed temperature used was 110° F.

to 10 psi., a point where there was insufficient steam to heat the incoming product to the desired 190° F. This low steam supply also decreased the velocity through the first chamber, as shown by time tests. Because of this, the product was held a sufficient length of time to result in good pasteurization below 185° F. This inherent safety feature of a reduced volume of steam resulting in an increased holding time also was demonstrated in the test results obtained using low preheat temperatures (table 6).

#### SUMMARY

As the amount of steam used in the Vacreator was reduced, the average time required for the test liquid (water) to pass through the first chamber was increased from 0.75 to 0.89 sec. Under set conditions, as the pump capacity was increased from 1,800 to 5,300 lb. per hour the time required for the test liquid to pass through the first chamber was decreased from 0.74 to 0.61 sec. The time required for complete travel through the Vacreator varied from 5.63 to 7.16 sec.,

depending upon the capacity at which the machine was operated. The 170° F. temperature ordinarily used in the second effect was found to be high enough to have considerable lethal effect.

If, from a Public Health angle in the case of mix pasteurization, it is desirable to require the temperature effect of the first chamber treatment to be the equivalent of or better than that obtained with pasteurization at 155° F. for 30 min., then, according to the results obtained, the temperature carried in the first effect should not be less than 194° F.

Dropping the preheating temperature from the standard temperature of 110 to as low as 49° F. did not alter the efficiency of pasteurization. When the in-feed pump speed was increased above the rated capacity of 3,000 lb. per hour, there was no change in the efficiency of pasteurization.

When the amount of the steam used in the first chamber was reduced from 500 lb. to approximately 200 lb. per hour, the efficiency of pasteurization remained the same. Partial clogging of the spray jets in the first chamber or removal of the spray pan did not result in a change in the effectiveness of the pasteurization process.

Overloading the Vacreator as much as 50 per cent above the rated maximum capacity did not result in unsatisfactory pasteurization of the mix.

Reducing the steam pressure to below that recommended for proper operation of the Vacreator reduced the velocity of the product passing through the first chamber so that there was sufficient time for proper pasteurization.

When the Vacreator is operated so that a minimum temperature of 194° F. is maintained in the first chamber, results will be obtained that will equal or better the Public Health protection afforded ice cream mix pasteurized at 155° F. for thirty min. in a sealed glass tube.

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## RELATIVE STORAGE QUALITIES OF FROZEN AND DRIED MILK

P. H. TRACY, JOHN HETRICK<sup>1</sup> AND WALTER S. KRIENKE<sup>2</sup>

*Department of Food Technology, University of Illinois, Urbana*

The storage of both fat and milk solids-not-fat in frozen form is commonly practiced by the dairy industry (9). The destabilizing effect of the continued storage at freezing temperatures upon the normal dispersion of the milk solids is one of the factors limiting the applications of this method to the storage of cream and condensed milk. Sugar sometimes is added to the milk solids to prevent churning of the fat and destabilization of the milk casein.

In a series of experiments reported by Babcock *et al.* (1-7), it was brought out that homogenized milk remained normal when frozen and stored at a constant temperature. Extremely low ( $-40^{\circ}$  F.) temperatures were found best. Homogenized milk frozen and stored in the frozen state was found to have the solids more concentrated in the bottom section. Homogenized milk that had been stored frozen was held at usual fluid milk storage temperatures without any more rapid deterioration than would be expected of regular homogenized milk. The addition of sodium citrate to homogenized milk before freezing and storage improved the storage life of the milk, and added ascorbic acid helped preserve the normal flavor of the milk. Homogenized milk was kept as long as 120 hr. before freezing without adversely affecting the keeping quality of the frozen product. These investigators also found that rotating homogenized milk while it was being frozen prevented a segregation of the milk solids but did not improve the keeping quality of the stored milk.

In 1944, Doan and Leeder (8) recommended a procedure for preparation of frozen condensed milk. They proposed a preheating temperature of  $180^{\circ}$  F. for 15 min., a 3 to 1 concentration of the milk, homogenization of at least 3000 lb. before condensing, limiting the air incorporation to 20 to 30 per cent when freezing with a continuous freezer, holding at a temperature not higher than  $-10^{\circ}$  F. during freezing, storage and dispensing, and reconstitution in  $180^{\circ}$  F. water. These authors claim the frozen condensed milk can be held for 10 to 12 wk. without effect upon the fat or protein if stored at  $-15$  to  $-20^{\circ}$  F.

### EXPERIMENTAL PROCEDURE

Two lots of fresh milk were used for these studies. The first experiment was begun in October, 1944, using milk obtained from a local cheese factory. No attempt was made to select milk of high quality. Upon being received at the University experimental plant, the milk was heated to  $85^{\circ}$  F. and clarified. It then was heated to  $140^{\circ}$  F. and homogenized at this temperature using a pressure of 2500 lb. per in.<sup>2</sup>. The homogenized milk then was pasteurized at  $170^{\circ}$  F. for 20 min. While it was realized that the high temperature used for pasteurizing

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<sup>1</sup> Now Research director, Dean Milk Co., Rockford, Ill.

<sup>2</sup> Now with the Dept. of Dairy Husbandry and Dairy Manufactures, University of Florida.

the milk would lower the flavor score of the fluid milk as well as the concentrated milk, it had been established in previous experiments by numerous investigators that if dried whole milk is not made from milk preheated to temperatures as high as 170° F. oxidized flavors are likely to develop in a short time after storage. It also was realized that the high heat treatment would retard or prevent the development of an oxidized flavor in the frozen milk (2).

A portion of the pasteurized milk was cooled to 40° F., filled into quart paper containers (American Can type) and frozen in the hardening room at  $-10 \pm 5^\circ$  F. The balance of the milk was cooled to 145° F. and condensed to a 11.5° Baume. A portion of the condensed milk was cooled to 40° F. and put into quart paper containers and frozen in an ice cream hardening room. Another batch of this condensed milk was frozen to a slush (27 to 28° F.) in a Creamery Package continuous ice cream freezer, packaged directly into quart paper milk containers and stored at the low temperature. To another portion of the condensed milk, 3 per cent by weight of dextrose was added; it was packaged and placed in the ice cream hardening room. The remainder of both the sweetened and unsweetened condensed milk was dried, using an experimental spray drier. A no. 72 nozzle and 1000 lb. spray pressure with a 140° F. spray temperature were used.

The dried milks were packed in no. 1 cans with a packing density of 0.5 g. per milliliter at 110° F. Half of the cans were air-packed and the remainder were nitrogen-packed. The nitrogen-packed samples were evacuated twice, one gassing following the other immediately. Half of the air- and nitrogen-packed samples of powder from each batch were stored in the hardening room with the frozen fluid and condensed samples. The other samples of powder were stored at room temperature. The room temperature was thermostatically controlled at 72° F. during the time the building was heated.

At intervals during storage, the powdered samples were gas-analyzed and all samples were reconstituted to the original milk composition before use. The frozen products were partially defrosted by submerging the quart paper containers in a water bath having a temperature of 110° F., which is just below the melting point of the paraffin coating the container. To the block of frozen condensed milk was added the amount of water (160° F.) required to restore the milk solids to their normal concentration. The mixture was agitated gently at intervals until thawed. Pre-thawing was necessary to remove the frozen block without having paraffin attached to it. So tenaciously was the paraffin attached that "peeling" of the carton resulted in transfer of a considerable portion of the paraffin from the carton to the frozen block and subsequent incorporation in the defrosted product. Shrinkage during storage of the frozen, condensed milk resulted in a similar removal of paraffin by the frozen block in the samples that had been pre-frozen in the ice cream freezer. Still-frozen samples did not shrink.

The reconstituted and thawed samples were examined for fat separation, curdy and flaky appearance and ascorbic acid content and were judged for flavor by at least three experienced milk judges using the value of 25 as a perfect score, 23 to 25 no criticism and anything under 12 as unsalable.

The second experiment was started in December, 1944, using University of

Illinois herd milk. The following procedure was followed in processing the milk: (a) clarified at 90° F.; (b) pasteurized at 170° F. for 20 min. (after clarification); (c) cooled to 145° F. in the vat and a portion homogenized at 2500 lb. pressure; (d) the remainder was condensed and then homogenized at 2500 lb. pressure at a temperature of 135° F.; (e) to a portion of the condensed milk was added 1.5 per cent dextrose; (f) the spray pressure used for drying the condensed milks was 500 lb.; and (g) the powder had a packing density of 0.484 g. per milliliter. The samples were stored and observations were made in a manner similar to those listed in the first experiment except that the condensed milk to which dextrose was added was divided into two lots. One lot was placed directly into paper containers before being placed in the hardening room and the second lot first was passed through a continuous freezer and reduced to a temperature of 27 to 28° F. before being placed in the paper containers.

The analytical data on the experimental samples when freshly prepared are given in tables 1 and 2 for experiments number 1 and 2, respectively.

TABLE 1

*Analytical data on the relative keeping quality of frozen milk, frozen condensed milk, and spray-dried whole milk powder  
(Experiment 1)*

Product	B.F.	T.S.	Acidity	Vit. C	Bact. count	Coli count	Copper	Iron	Initial oxygen	Mois- ture
	(%)	(%)	(%)	(mg./l.)			(ppm)	(ppm)		(%)
Standardized milk	3.5	12.05	0.155	15.4	14,200	12	0.10	0.51		
Pasteurized milk				13.3	600	0				
Condensed milk	12.65	43.22	0.55	9.8	760	0	0.40	2.77		
Condensed milk plus 3% dextrose	12.33	45.17			850	8				
Powdered whole milk	28.88	98.0	0.14*		600*		1.43	7.65	0.95	2.0
Powdered milk (Dextrose added)	27.53	97.2	0.135*		1000*		1.40	7.40	0.94	2.8

Powder sample identification  
Experiment 1

Cond. no.	Product dried	Sample no.	Type of pack	Symbol used	Storage temp.	Symbol used
(1)	Condensed milk	100	Air	A	Room	R
		101	Nitrogen	N	Room	R
		102	Air	A	-10° ± 5° F.	
		103	Nitrogen	N	-10° ± 5° F.	
(2)	Condensed milk and 3% Dextrose	104	Air	A	Room	R
		105	Nitrogen	N	Room	R
		106	Air	A	-10° ± 5° F.	
		107	Nitrogen	N	-10° ± 5° F.	

\* After reconstitution.

TABLE 2

*Analytical data on the relative keeping quality of frozen, frozen condensed and spray-dried whole milk powder*  
(Experiment 2)

	B.F.	T.S.	Acidity	Vit. C	Copper	Iron	Initial oxygen	Moisture
	(%)	(%)	(%)	(mg./l.)	(ppm)	(ppm)		(%)
Standardized milk	3.5	12.6	0.16	16.4				
Pasteurized milk				11.7				
Condensed milk	11.5	41.97	0.155*	10.6*				
Powdered milk	27.39	98.5	0.15*	8.6*	1.50	3.3		1.50
Powdered milk (dextrose added)	26.52	98.0	0.15	8.0	1.42	2.8		2.0

Powder sample identification  
Experiment 2

Cond. no.	Product dried	Sample no.	Type of pack	Symbol used	Storage temp.	Symbol used
(3)	Condensed milk	136	Air	A	Room	R
		137	Nitrogen	N	Room	R
		138	Air	A	Hardening room	HR
		139	Nitrogen	N	Hardening room	HR
(4)	Condensed milk and 1.5% dextrose	140	Air	A	Room	R
		141	Nitrogen	N	Room	R
		142	Air	A	-10° ± 5° F	
		143	Nitrogen	N	-10° ± 5° F	

\* After reconstitution.

At intervals during the storage period, thawing observations, ascorbic acid determinations and flavor scores were made on the thawed products; analyses of the headspace gas of nitrogen-packed powder were made and ascorbic acid values and flavor scores were determined on the reconstituted powdered milk, both air- and nitrogen-packed. In the first experiment, sixteen periodic observations were made over a storage period of 523 days (approximately 17 mo.). In the second experiment, the milk samples were stored 365 days and twelve periodic observations were made.

#### EXPERIMENTAL RESULTS

The data on the thawing observations of the frozen products, the oxygen content of the powdered milk and the flavor scores and ascorbic acid values of both types of products are given in tables 3 and 4. At the beginning of the storage period, the score of the reconstituted condensed milk was higher than that of pasteurized homogenized fluid milk and that of the reconstituted powdered milk was higher than the flavor score of the reconstituted condensed milk. These samples had much less cooked flavor as a result of the condensing and drying opera-



TABLE 3 (continued)

Powdered whole milk samples				Storage time <sup>b</sup> (days)															
Sample no.	Made from cond. no.	Type Where stored	Analysis	Initial	23	48	79	105	140	175	198	227	269	307	338	364	425	464	523
100	1	A B	Flavor score Flavor criticism Vit. C. (mg./l.)	22.0	22.0	19.5	19	17.0	16.5	16.0	15.0	15.0	10.	10.0	0	0			
						SI.Ox. 5.9	Ox. 3.1	Ox. 4.0	Ox. 3.6	Ox. 4.4	Ox. 4.4	Ox. 3.6	Ox. 3.2	Ox. 3.6	Ox. 3.3	Ox. 3.5			
101	1	N B	Flavor score Flavor criticism Vit. C. (mg./l.) % O <sub>2</sub>	22.0	22.0	20.0	19.5	18.5	18.5	18.0	18.0	18.0	17.0	16.0	13.0	10.0	8.0	15	
				.95	1.92	1.70	2.22	1.46	1.31	1.33	1.33	1.21	.84	.96	1.09	2.11	.68	.93	.86
102	1	A HR	Flavor score Flavor criticism Vit. C. (mg./l.)	22	22	21.5	21.5	20.0	20.0	17.0	19.0	14.0	17.0	16.5	14.0				
						LF 8.0	7.5	7.0	5.2	SLM. 5.9	SLM. 8.4	7.8	6.8	7.8	M 7.4	M			
103	1	N HR	Flavor score Flavor criticism Vit. C. (mg./l.) % O <sub>2</sub>	22.0	22.0	22.0	22.0	21.0	20.5	20.0	20.0	19.5	19.5	18.0	17.5	18.0	17.5	17.5	12.0
						LF 8.0	7.7	7.0	5.7	F 6.9	LF 8.4	8.3	7.4	7.8	M 7.4	M 8.0	7.1	7.5	6.5
				.95	1.69	1.93	1.63	2.12	1.94	2.15	2.18	1.98	1.96	1.92	.95	2.03	2.15	1.94	2.18
104	2	A B	Flavor score Flavor criticism Vit. C. (mg./l.) % O <sub>2</sub>	22.0	22.0	20.5	20.0	19.0	17.5	17.0	16.5	14.0	15.0	13.0	3.0	0	0	10	10
						SLS 6.4	5.1	4.0	4.1	Ox. 4.4	Ox. 4.9	Ox. 4.7	Ox. 4.2	Ox. 7.6	Ox. 3.7	Ox. 4.0	Ox. 2.7	Ox. 3.5	Ox. 1.7
105	2	N B	Flavor score Flavor criticism Vit. C. (mg./l.) % O <sub>2</sub>	22.0	22.0	20.5	20.5	18.5	19.0	19.0	18.5	17.5	17.0	17.0	14.5	13.5	16.0	8.0	17.0
						SLS 7.0	SI.Ox. 5.1				Ox. 7.1	Ox. 6.8	Ox. 6.8	Ox. 7.1	Ox. 7.0	Ox. 7.5	Ox. 5.8	Ox. 6.5	Ox. 6.1
				.94	1.57	1.93	1.90	1.55	1.35	1.29	.99	.84	.53	2.23	1.34	.90	1.20	1.20	.80
106	2	A HR	Flavor score Flavor criticism Vit. C. (mg./l.)	22.0	22.0	21.5	21.0	20.5	20.5	20.0	17.0	17.0	13.0	16.0	14.0	15.0	14.0		14.0
						7.0	7.1	7.0	3.6	M 6.9	M 8.0	M 6.2	M 6.8	M 7.5	M 7.4	M 7.5	M 6.7	M 6.5	
107	2	N HR	Flavor score Flavor criticism Vit. C. (mg./l.) % O <sub>2</sub>	22.0	22.0	21.5	21.0	20.0	21.0	19.0	18.5	19.5	19.0	17.5	17.0	17.0	17.0	18.0	16.0
						7.5	7.1	7.0	5.7	7.4	M 8.0	M 7.8	M 7.4	M 7.5	M 7.4	M 7.5	M 6.2	M 7.5	M 6.5
				.94	1.45	1.58	1.21	1.56	1.51	1.73	1.64	1.19	1.46	1.26	1.45	1.48	1.78	1.67	1.50

TABLE 4

*The flavor, ascorbic acid, thawing observations and oxygen content of fluid,<sup>a</sup> concentrated and powdered whole milk on storage. (Experiment 2)*

Sample	How Frozen	Analysis	Initial	Storage time <sup>b</sup> (days)											
				22	48	92	119	148	176	210	248	280	306	365	
Pasteurized and homogenized	HR	Flavor score		19.5	20.0	19.0	18.5	18.0	18.0	18.0	19.5	19.0	19.0	19.5	
		Flavor criticism		C	C	C	C	C	C	C	C	C	C	C	C
		Vit. C. (mg./l.)	11.7	9.2	9.0	8.1	8.5	8.2	9.2	9.0	9.6	9.9	10.0	7.6	
		Thaw Obs.													
Condensed 3.5-1	HR	Flavor score		21.5	21.0	20.0	19.5	19.0	19.0	19.0	19.5	19.5	19.5	20	
		Flavor criticism													
		Vit. C. (mg./l.)	10.6	8.7	8.3	7.2	7.6	5.9	8.4	5.1	5.9	8.6	8.3	7.1	
		Thaw Obs.													
Condensed 3.5-1	F	Flavor score		21.5	21.0	20	19.5	19.5	19.5	19.5	20.0	20.0	20.0	19.75	
		Flavor criticism													
		Vit. C. (mg./l.)	10.6	9.4	7.5	7.1	7.3	5.6	8.2	5.4	6.6	9.9	7.6	7.6	
		Thaw Obs.													
Condensed 3.5-1	HR	Flavor score		21.5	21.0	18.5	20.0	20.0	20.0	19.0	19.5	19.5	19.5	20.5	
		Flavor criticism		SlC	C	C	C	C	C	C	C	C	C	C	
		Vit. C. (mg./l.)		8.1	7.6	8.0	7.8	6.8	9.2	4.6	5.8	10.7	8.0	7.0	
		Thaw Obs.													
Milk condensed 3.5-1 and 1.5% dextrose added	F	Flavor score		21.5	21.0	18.5	20.0	20.0	20.0	19.0	19.5	19.5	19.5	20.25	
		Flavor criticism													
		Vit. C. (mg./l.)	---	9.3	7.8	7.7	7.0	6.1	8.4	5.1	6.6	10.2	7.4	8.2	
		Thaw Obs.													

<sup>a</sup> Raw milk scored 19 in flavor and contained 16.4 mg./l. vitamin C.

<sup>b</sup> Concentrated Milk Samples (stored at  $-10^{\circ} \pm 5^{\circ}$  F.)

<sup>c</sup> See table 3.

TABLE 4 (continued)

Powdered whole milk samples					Storage time ( <i>days</i> )											
Sample no.	Cond. no.	Type	Where stored	Analysis	Initial	22	48	92	119	148	176	210	248	280	306	365
136	3	A	R	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> )	8.6	Sl.Ox. 7.1	19.0 6.0	18.5 5.1	17.5 5.5	15.5 5.5	15.0 5.0	15.0 3.7		9.0 Ox. 5.1		
137	3	N	R	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> ) % O <sub>2</sub>	8.6	Sl.Ox. 7.7 1.81	19.5 6.5 1.79	19.0 7.1 1.44	19.0 7.0 1.65	17.0 7.7 1.45	16.5 6.7 1.09	16.5 7.4 .72	15.0 7.5 .85	15.0	12.0 Ox. 5.1	
138	3	A	HR	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> )	8.6	C 6.6	20.5 7.5	20.0 7.6	20.0 8.0	20.0 8.2	20.0 7.6	20.0 8.0	19.0 8.2	18.5 7.9	18.5 8.0	16.5 6.7
139	3	N	HR	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> ) % O <sub>2</sub>	8.6	C 7.7 1.44	20.5 8.0 1.52	20.0 8.1 1.59	20.0 8.0 1.47	20.0 8.2 1.60	20.0 7.6 1.20	20.0 8.0 1.11	19.0 8.6 1.50	18.5 7.9 1.74	18.5 8.0 1.98	17.5 7.1 1.69
140	4	A	R	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> )	8.0	Sl.Ox. 7.1	19.0 5.0	19.0 5.6	18.0 6.0	16.5 5.9	17.5 5.0	17.5 4.8				
141	4	N	R	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> ) % O <sub>2</sub>	8.0	Sl.Ox. 7.1 1.26	19.5 6.5 1.36	18.5 7.1 1.14	18.5 7.5 .87	18.0 7.7 1.14	18.0 7.6 .78	18.0 8.0 .45	16.0 7.5 .85	15.0	15.0 Ox. 8.5	.63
142	4	A	R	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> )	8.0	C 7.7	21.0 6.5	21.0 7.6	20.5 8.0	19.0 8.2	18.0 7.6	18.0 8.0	16.0 8.2	16.0	16.5 M	
143	4	N	HR	Flavor score Flavor criticism Vit. C( <i>mg./l.</i> ) % O <sub>2</sub>	8.0	C 8.2 1.03	19.5 6.5 1.16	18.0 7.6 1.28	20.0 8.0 1.17	19.5 8.2 1.30	18.5 7.6 1.20	19.0 8.0 1.13	18.0 8.2 1.31	18.0	18.0 M	17.0 S 7.6 1.42



tions, indicating that at least a portion of the substances responsible for cooked flavor are volatile.

Fluid or condensed milks can be kept frozen at low temperatures for considerable lengths of time without showing any curdy, oily or flaky appearance upon defrosting (tables 3 and 4). In the second experiment (table 4), at the end of a year of storage, the fluid and condensed milk still defrosted satisfactorily. In the first experiment (table 3) there was normal thawing and reconstitution up to 364 days of storage when the condensed milk frozen in the freezer had a slightly curdy appearance on reconstitution. The next sample to show a curdy appearance was the sweetened condensed milk frozen in the freezer, which was curdy and showed fat separation when examined on the 425th day. This condition in these two samples became progressively worse for the duration of the study. The pasteurized fluid milk showed a slight curdy appearance at the end of 1 yr. The condensed milk frozen in the quiescent state in the ice cream hardening room had a satisfactory appearance even after 523 days of storage. However, the frozen condensed milk was particularly sensitive to heat-shocking. Samples brought out to room temperature and then returned to the hardening room showed a destabilized condition of the milk proteins in a few days. Similar effects were produced when the frozen milk was transferred from the sub-zero temperature to one slightly above 0° F.

The greatest reduction in ascorbic acid values came as a result of the heating that occurred during processing. The amount of ascorbic acid retained in the thawed frozen products was only slightly less than it was when the products were first stored. The concentration of milk solids, the addition of dextrose or the method of freezing did not seem to influence to any extent the retention of ascorbic acid on storage. Although initially powdered whole milk will show less ascorbic acid content, the retention of ascorbic acid in the nitrogen-packed product compared favorably with that of the frozen products. In the milk powder samples, greater ascorbic acid loss occurred in the air-packed than in the nitrogen-packed samples and more loss of ascorbic acid occurred in those stored at room temperature than in those stored at  $-10^{\circ} \pm 5^{\circ}$  F.

The frozen fluid milk and frozen concentrated milks possessed better flavor keeping qualities than the gas-packed whole milk powder made from the same lot of milk and stored at the same temperature. As the storage period advanced, the powder stored in the hardening room usually became progressively metallic or oxidized while the frozen milk and concentrated milk lost their fresh milk flavor but remained highly palatable. The flavor of the frozen products remained satisfactory throughout the storage period of 523 days in the case of experiment 1 and for 1 yr. in the case of experiment 2, while the gas-packed whole milk powder stored at the same temperature became unsatisfactory after a storage period of 307 days for experiment 1 and approximately 280 days for experiment 2. The air-packed powder stored at  $-10 \pm 5^{\circ}$  F. possessed a metallic flavor after 175 days storage in experiment 1 and 176 days of storage in experiment 2. When powder was stored at room temperature, an oxidized flavor was observed after 198 days of storage in the nitrogen-packed samples of experiment 1 and 176 days

in the second experiment. At room temperature the air-packed samples became unsatisfactory at 105 days and 119 days for the two experiments, respectively.

After thawing, the frozen milks in some instances were held at 40° F. for 72 hr. yet they did not change in flavor.

The concentration of the milk (approximately 3.5 to 1), the addition of dextrose prior to freezing or drying, the method of freezing (slow freezing at  $-10 \pm 5^\circ$  F. vs. freezing initially to a slush in a continuous ice cream freezer) did not prove to be important factors in flavor changes, ascorbic acid retention or stability of the milk solids on thawing of the frozen products.

#### CONCLUSIONS

Fluid milk or milk concentrated approximately 3.5 to 1 can be satisfactorily stored at a uniformly low temperature ( $-10 \pm 5^\circ$  F.) for at least 1 yr. Milk concentrated approximately 3.5 to 1 or fluid milk can be stored in frozen state for 1 yr. with less flavor change than the same milk stored at the same temperature in dried form (gassed or ungassed).

Condensing before freezing or the addition of dextrose to the milk did not prove to be important factors in storing milk in a frozen state either from the standpoint of flavor changes or changes in the physical state of the milk proteins.

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# MOTILITY OF BOVINE SPERMATOZOA AND CONTROL OF BACTERIA AT 5 AND 25° C. IN EXTENDERS CONTAINING SULFANILAMIDE, PENICILLIN, STREPTOMYCIN AND POLYMYXIN

R. H. FOOTE AND R. W. BRATTON

*Laboratory of Animal Breeding and Artificial Insemination  
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.*

In artificial breeding the general practice is to preserve bovine spermatozoa in a buffered yolk medium by cooling to approximately 5° C. and holding the extended semen at that temperature until used. This procedure is based on considerable experimental evidence which has been reviewed by Anderson (1) showing that spermatozoa survive longer at 5° C. than at higher temperatures. Considerable expense is involved in packaging and storing semen so as to maintain a temperature of 5° C. until the semen is used for insemination. Therefore, any method of preserving the spermatozoa which is cheaper than refrigeration is of practical importance. Foote and Salisbury (5, 6) have shown that the motility of spermatozoa stored at 20° C. in a citrate-phosphate buffer is prolonged by the addition of a number of antibacterial agents. Since egg yolk is an excellent medium for bacterial growth, one of the problems of preserving spermatozoa in egg yolk at "room" temperature appears to be that of controlling bacterial growth at this temperature. Dimitropoulos *et al.* (2) and Hennaux *et al.* (7, 8) have reported that antibacterial agents were beneficial in preserving the motility of spermatozoa extended with citrate-yolk and incubated at 37° C. The present paper is a report of comparisons of spermatozoan motility and bacterial growth in extended semen when it is stored at 5° C. and at 25° C. in extenders containing various antibacterial agents.

## EXPERIMENTAL PROCEDURE

The basic medium employed for extending the semen consisted of egg yolk mixed with an equal amount of buffer containing 3.6 g. sodium citrate dihydrate per 100 ml. of water redistilled in glass. Based on experiments previously reported by Foote and Bratton (4), five different extenders were prepared by adding the following amounts of antibacterial agents per milliliter of basic extender: (a) 3 mg. sulfanilamide, (b) 500 Oxford Units of crystalline sodium penicillin G, (c) 500  $\gamma$  (units) of streptomycin base ( $\text{CaCl}_2$  complex), (d) 500  $\gamma$  equivalents of polymyxin B sulfate<sup>1</sup> and (e) a combination of a, b, c and d. In addition, the basic extender was included as a control. Thus, with six extenders and two storage temperatures, 5 and 25° C., a total of 12 treatments were involved. To accomplish a simultaneous comparison of all 12 treatments, each semen sample was divided into six equal portions, and each portion extended with one of the six extenders to give approximately  $15 \times 10^6$  motile spermatozoa per milliliter of

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<sup>1</sup> "Aerosporin" brand polymyxin B was kindly supplied by D. S. Searle of the Burroughs Wellcome and Co., Inc., Tuckahoe, New York. The amount used was equivalent to 500  $\gamma$  of pure standard.

TABLE 1

*The counts of bacteria in semen before storage and after 24 hr. of storage at 5 and 25° C. in citrate-yolk extender with and without sulfanilamide and antibiotics*

Sample	Semen as col- lected	After ex- tension of semen	After 24 hr. of storage in extenders containing:											
			No antibac- terial agent		Sulfa- nilamide		Penicillin		Streptomycin		Polymyxin		All antibac- terial agents	
			5° C.	25° C.	5° C.	25° C.	5° C.	25° C.	5° C.	25° C.	5° C.	25° C.	5° C.	25° C.
(1,000's of bacteria/ml.)														
1	33.0	0.6	2.8	120	2.4	28.0	0.0	0.1	0.0	86.0	1.4	2.0	0.0	0.0
2	61.0	1.1	1.7	26,000	1.2	1.0	0.2	1.0	0.1	1.0	0.7	1.0	0.0	1.0
3	71.0	0.7	1.4	110,000	0.9	24.0	0.7	30,000.0	0.5	2,000.0	1.1	30,000.0	0.3	0.0
4	0.7	0.01	0.5	31	0.5	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
5	0.4	< 0.01	0.6	21	0.3	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	130.0	1.0	3.2	57,000	3.3	3.0	0.0	1.0	0.2	0.0	2.9	1.0	0.0	0.0
7 <sup>a</sup>	160.0	1.7	9.4	49,000	8.9	27.0	0.8	64.0	0.4	69.0	2.4	22.0	0.3	0.0
8	59.0	1.4	7.7	51,000	6.7	11.0	0.0	0.0	0.1	0.0	3.2	1.0	0.0	0.0
9 <sup>a</sup>	13.0	0.2	0.5	410,000	0.5	6.6	0.2	800.0	0.0	0.0	0.5	0.0	0.0	0.0
10 <sup>a</sup>	110.0	1.5	5.6	800,000	7.1	460.0	2.3	100,000.0	7.6	30,000.0	0.0	0.0	0.0	0.0
11 <sup>a</sup>	140.0	1.6	3.0	27,000	0.1	0.0	2.7	1,800.0	0.0	1.0	0.0	12.0	0.0	0.0
12 <sup>a</sup>	28.0	0.3	1.9	81,000	1.5	2.4	0.0	25,000.0	0.0	4.3	0.2	30,000.0	0.0	0.0
13	9.0	0.08	0.9	110	0.9	4.0	0.1	40.0	0.2	2.0	0.3	1.0	0.0	0.0
14 <sup>a</sup>	31.0	0.3	3.4	790,000	3.3	5.8	3.0	30,000.0	3.8	50,000.0	0.0	0.0	0.0	0.0
15	100.0	1.0	20.0	84,000	12.0	18.0	0.2	0.0	0.6	370.0	10.0	1,000.0	0.0	0.0
16	31.0	0.3	7.3	240,000	1.7	1.5	0.2	0.2	0.1	0.0	0.3	510.0	0.0	0.0
17	8.0	0.08	1.0	4	0.9	0.0	0.0	1.0	0.1	0.0	1.2	0.0	0.0	0.0
18	500.0	11.0	39.0	45,000	57.0	35.0	2.1	5.0	0.6	2.0	21.0	10.0	0.1	0.0
Av.	83.0	1.3	6.1	150,000	6.1	35.0	0.7	10,000.0	0.8	4,600.0	2.5	3,400.0	0.04	0.06

<sup>a</sup> Bacterial flora included *Pseudomonas* type organisms.

extended semen, the average extension rate being about 1 to 70. From each of these extended portions, duplicate sub-samples were taken so that the six extenders could be stored at 5 and 25° C. The sub-samples were examined microscopically for the per cent of motile spermatozoa and the rate of progressive movement after 2, 24 and 72 hr. of storage. This extension and storage procedure was replicated with 18 samples of high quality semen obtained from 18 bulls in the active stud of the New York Artificial Breeders' Cooperative, Inc.

The approximate number of living bacteria present in each semen sample immediately following collection and in all samples of extended semen after 24 hr. of storage was determined by the plate count method as employed in this laboratory (4). The 24-hr. period of storage was chosen as the most desirable time to estimate the numbers of bacteria present because most bovine semen is used for insemination at about this time.

The statistical significance of the differences between the average per cent of motile spermatozoa in the different extenders stored at the two temperatures was tested by analysis of variance (9).

#### RESULTS

The number of bacteria present in the freshly collected semen, in the unstored extended semen and in the extended semen stored for 24 hr. is shown in table 1. Most of the freshly collected semen samples had a bacterial count of less than 120,000 per milliliter. After the semen was mixed with nearly sterile extender so as to contain approximately 15 million motile spermatozoa per milliliter, the bacterial counts, with the exception of semen sample 18, were reduced to less than 2,000 per milliliter, with sample 5 having a count of less than 10 per milliliter. After 24 hr. of storage at 5° C., bacterial growth was not excessive in any of the extended semen samples. Usually, when used separately, each of the antibiotics and sulfanilamide partially inhibited bacterial growth. The combination of antibiotics with sulfanilamide usually inhibited bacterial growth completely.

At 25° C. bacteria multiplied rapidly when no antibacterial agent was present. Sulfanilamide consistently reduced bacterial growth. Penicillin, streptomycin and polymyxin were highly bactericidal when the predominating types of bacteria were sensitive to the particular drug present. In samples 10 and 14, *Pseudomonas pyocyaneus* (usually sensitive to streptomycin) thrived at 25° C. in the presence of streptomycin, but was inhibited completely by polymyxin. Again the combination of antibiotics with sulfanilamide was highly bactericidal.

Microscopic examinations within 2 hr. after the semen was extended indicated that the percentages of motile spermatozoa were similar for all treatments. However, after 24 and 72 hr. of storage, large differences existed which are evident in table 2. At 5° C. the per cent of motile spermatozoa was similar in all extenders throughout the 72-hr. storage period. At 25° C. after 24 and 72 hr. of storage, the combination of sulfanilamide plus antibiotics was more effective ( $P < 0.05$ ) in preserving the motility of the spermatozoa than was penicillin, streptomycin or polymyxin. While penicillin, streptomycin and polymyxin did not differ from each other, each was superior to no antibiotic at this temperature. At 25°

TABLE 2

*The per cent of motile spermatozoa in extenders containing different antibacterial agents and stored at 5 and 25° C. (Av. of 18 ejaculates)*

Length of storage	Temperature	Extenders containing:					
		No antibacterial agent	Sulfanilamide	Penicillin	Streptomycin	Polymyxin	All antibacterial agents
(hr.)	(° C.)	(Percentage of motile spermatozoa)					
24	5	63	62	63	62	63	62
	25	41	54	49	43	46	58
72	5	56	55	56	56	57	53
	25	8	34	17	17	23	39

C. spermatozoan motility generally was poorer than at 5° C. but at the 24-hr. storage interval the difference between the per cent of motile spermatozoa at these two temperatures in extenders containing sulfanilamide plus antibiotics was not significant statistically ( $P > 0.05$ ).

#### DISCUSSION

The results of these experiments indicate that bovine semen in extenders containing sulfanilamide, penicillin, streptomycin and polymyxin can be stored for at least 24 hr. at 25° C. with much of the deleterious effect of high temperatures eliminated by the inhibition of bacterial growth. Sulfanilamide alone was nearly as effective in maintaining the motility of the spermatozoa as was the combination of sulfanilamide and antibiotics, but bacterial growth was more completely inhibited by the combination. Frequently, the antibiotics used alone were more bactericidal than sulfanilamide used alone, but the percentage of motile spermatozoa was higher when sulfanilamide was present.

Putrefaction of the egg yolk consistently occurred when bacterial growth was high. The percentage of samples exhibiting a putrid odor after 72 hr. of storage at 25° C. was 72, 6, 11, 22, 17 and 0, respectively, for the extenders containing no antibiotics, sulfanilamide, penicillin, streptomycin, polymyxin and the combination of sulfanilamide and antibiotics. The products of egg yolk putrefaction may have been spermicidal. The pH of all samples after 72 hr. of storage at 5° C. was approximately 6.70, while at 25° C. it was 6.50. This small difference would appear to eliminate H-ion concentration as an important factor in reducing spermatozoan motility at the higher temperature.

Fertility data obtained by Drake (3) indicate that semen processed and stored without refrigeration may be used for insemination in cool weather (April) but is not satisfactory for use in warm weather (July). The poorer results in July may have been caused by the direct deleterious effects of high temperatures on the spermatozoa or the citrate-yolk extender, since the bacteriological data presented in this paper indicate that bacterial growth is effectively inhibited at 25° C. by a combination of sulfanilamide and antibiotics. As a consequence of this control of bacterial growth in extenders stored unrefrigerated, the problem of prolonging the motility and fertility of spermatozoa in unrefrigerated extenders now appears to be one of achieving sufficient control of spermatozoan metabolism.

## SUMMARY

The feasibility of storing bovine semen at 25° C. for use in artificial insemination to eliminate the expense of refrigerating the semen at 5° C. was investigated. Sulfanilamide, penicillin, streptomycin, polymyxin and a combination of these were added to 3.6 per cent citrate-yolk extender. The citrate-yolk extender containing no sulfanilamide or antibiotics served as the control. Eighteen semen samples were stored in each of the six extenders at 5° C. and at 25° C. The per cent of motile spermatozoa after 24 hr. of storage was lower when the semen was stored at 25° C. than when it was stored at 5° C. except in the extender containing the combination of antibacterial agents. In nearly all samples, this combination of sulfanilamide and antibiotics completely inhibited bacterial growth at both temperatures. This combination of antibacterial agents gives promise of making possible the development of an extender for bovine semen which will not require refrigeration.

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# RELATIONSHIP OF HYALURONIDASE CONCENTRATION TO FERTILITY OF DAIRY BULL SEMEN<sup>1</sup>

JAMES E. JOHNSTON<sup>2</sup> AND JOHN P. MIXNER  
*New Jersey Agricultural Experiment Station, Sussex*

The possibility that the concentration of the enzyme hyaluronidase in mammalian semen is a critical factor in fertility has led the authors to investigate this relationship in dairy bulls.

In fertility studies in rabbits, Rowlands (8) found that by adding seminal plasma from killed spermatozoa suspensions containing hyaluronidase to dilute spermatozoa suspensions the median effective spermatozoa concentration for fertility was reduced to one-sixth of that of the controls. In similar experiments, however, Chang (1) indicated that the increased fertilizing capacity obtained by adding seminal plasma to dilute suspensions of spermatozoa was not due to hyaluronidase but to some other seminal plasma factor. Seminal plasma in which the hyaluronidase had been inactivated by heat was similarly effective, whereas added bull testes hyaluronidase had no effect.

Kurzrok *et al.* (5) reported that in six cases of human female infertility where the female was apparently not at fault and where the male seminal hyaluronidase concentration was low, the application of bull testes hyaluronidase to the uterine cervix with subsequent coitus resulted in pregnancies. Later, Kurzrok (4) reported that 33 out of 102 similar clinical patients conceived following the application of bull testes hyaluronidase to the uterine cervix. Entirely negative results were obtained by Seigler (10) in a series of 48 cases of human female infertility where hyaluronidase was applied and where the female presumably was not at fault. Semen samples from the male partner were not assayed for hyaluronidase in these cases.

A zero order correlation coefficient of  $-0.32$  (significant at the 5 per cent level) between hyaluronidase titer and fertility of dairy bull semen was obtained by Sallman and Birkeland (9). The hyaluronidase assays in this case were made within 20 hr. of the time of ejaculation. In considering the negative correlation which they obtained, these authors suggested that the removal of hyaluronidase from semen might improve the fertility of the semen. In this general connection it is interesting to note that Johnston and Mixner (3) found a first order partial correlation (limiting effect of sperm concentration) of  $-0.30$  (significant at the 5 per cent level) between percentage of live spermatozoa in dairy bull semen and hyaluronidase concentration.

## METHODS

One hundred and eighty-seven semen samples were obtained from 24 Guernsey and Holstein bulls at the bull stud of the Dairy Research Farm, New Jersey Agri-

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<sup>2</sup> Present address: Dairy Department, Louisiana State University, Baton Rouge.



cultural Experiment Station, Sussex, from November, 1947, to July, 1949. Semen samples were diluted for use at a rate not exceeding 1 to 100 and usually much less. Inseminations were made by the technicians of the Sussex County Co-operative Breeding Association, Inc. Fertility estimates were based on the percentage of non-returns to service, after a 60- to 90-day period following service, to first and second service cows.

Seminal hyaluronidase was assayed by the turbidimetric method, as outlined by Leonard *et al.* (6) and modified by Mixner and Johnston (7). Hyaluronidase potencies are expressed as milligrams equivalent to a standard preparation of bull testes hyaluronidase<sup>3</sup> (30 TRU per mg.). The Klett-Summerson photoelectric colorimeter with red no. 66 filter was used to measure turbidity. Two hyaluronidase assays were made upon the seminal plasma of each semen sample, the first assay being made within 1 hr. after the collection of the semen and the second assay being made after 24 hr. of incubation at 37° C. under toluene. These assay periods were chosen after a consideration of the scheme of development of hyaluronidase in bull semen (Johnston and Mixner, 2). Both of these measures of hyaluronidase concentration in semen were correlated with the fertility data.

The data on the semen samples were classified into three groups for purposes of calculation according to the number of first- and second-service cows bred to each sample. The groups are as follows: (a) 10-19 cows bred per sample, including 82 samples from 21 bulls for a total of 1,086 breedings, (b) 29 or more cows bred per sample, including 105 samples from 18 bulls for a total of 3,568 breedings, and (c) summation groups a and b, comprising 187 samples from 24 bulls to which 10 or more cows were bred per sample for a total of 4,654 breedings.

#### RESULTS AND DISCUSSION

The mean values, standard deviations and ranges for initial and 24-hr. hyaluronidase levels and for fertility are given in table 1 for each of the semen sample groups.

TABLE 1

*Means, standard deviations and ranges for fertility, initial and 24-hr. hyaluronidase levels for each semen sample group*

Character	Mean and standard error	Standard deviation	Range
(a) 10-19 cow bred/sample			
Initial hyaluronidase (mg./ml.)	45.3 ± 2.1	18.7	14-104
24-hr. hyaluronidase (mg./ml.)	119.0 ± 4.6	41.4	47-208
Fertility (% 60- to 90-day non-returns)	65.8 ± 1.5	13.9	37-92
(b) 20 + cows bred/sample			
Initial hyaluronidase (mg./ml.)	41.0 ± 1.7	17.4	14-98
24-hr. hyaluronidase (mg./ml.)	110.3 ± 4.1	41.4	44-246
Fertility (% 60- to 90-day non-returns)	66.4 ± 1.1	11.2	36-93
(c) 10 + cows bred/sample (groups a and b)			
Initial hyaluronidase (mg./ml.)	42.9 ± 1.3	18.1	14-104
24-hr. hyaluronidase (mg./ml.)	114.1 ± 3.0	41.6	44-246
Fertility (% 60- to 90-day non-returns)	66.1 ± 0.9	12.4	36-92

<sup>3</sup> The bull testes hyaluronidase was furnished through the courtesy of Schering Corp., Bloomfield, N. J.

Analysis of variance of the data revealed that there were highly significant differences among bulls with respect to initial and 24-hr. hyaluronidase levels and, also, with respect to the fertility estimation on each of the semen sample groups.

It is interesting to note (see table 1) the marked increase in hyaluronidase concentration of the seminal plasma which occurs between the time of the initial assay and the 24-hr. assay. This increase is of the order of 166 per cent and apparently represents the release of hyaluronidase by dying and dead spermatozoa into the seminal plasma (Johnston and Mixner, 2).

To determine whether any relationships existed between either of the measures of hyaluronidase concentration and fertility, zero order coefficients of correlation were calculated for each semen sample group of data on a "total," "between bull" and "within bull" basis (table 2). None of these correlations achieve statistical significance.

TABLE 2

*Zero order coefficients of correlation between initial and 24-hr. hyaluronidase levels and fertility estimates*

Semen sample group	No. semen samples	Initial hyaluronidase			24-hr. hyaluronidase		
		Total	Between bull	Within bull	Total	Between bull	Within bull
10-19 cows bred	82	+ 0.16	+ 0.42	- 0.12	+ 0.01	+ 0.32	- 0.22
20 + cows bred	105	- 0.17	- 0.24	- 0.12	- 0.08	- 0.01	- 0.12
10 + cows bred	187	0.00	+ 0.08	- 0.07	- 0.03	+ 0.10	- 0.11

Highly significant relationships have been shown to exist between hyaluronidase levels and the two factors, spermatozoa concentration and percentage of live spermatozoa (Johnston *et al.*, 3). Also, a significant relationship exists between these latter two factors and fertility (Stone *et al.*, 11). However, since none of the coefficients of correlation between seminal plasma hyaluronidase levels and fertility achieved statistical significance, further statistical manipulation of the data did not seem justified.

The bulls used in this study generally would be considered highly fertile. However, bulls occasionally have been eliminated from the breeding stud because of lowered fertility. There has been no indication in any case of a change in hyaluronidase levels associated with this lowered fertility.

#### SUMMARY AND CONCLUSIONS

The possibility that hyaluronidase is a critical factor in the fertility of dairy bull semen was investigated. One hundred and eighty-seven semen samples were collected from 24 dairy bulls. Hyaluronidase assays on seminal plasma were made initially and after 24 hr. of incubation at 37° C. under toluene on all samples. Semen samples were classified for statistical analysis into three groups according to the number of first- and second-service cows bred per sample: (a) 10 to 19 cows bred; (b) 20 or more cows bred; and (c) total of a and b. Coefficients of correlation were obtained between fertility estimates and hyaluronidase con-

centrations in each group on a "total," "between bull" and "within bull" basis. None of these coefficients of correlation attained statistical significance. From these results it seems doubtful if any significant relationship exists between the hyaluronidase and fertility levels of semen from bulls of relatively high fertility when the semen is diluted at a rate of 1 to 100 or less.

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# COMPARATIVE FERTILITY OF DILUTED BULL SEMEN TREATED WITH CALCIUM CHLORIDE COMPLEX STREPTOMYCIN OR DIHYDRO STREPTOMYCIN SULFATE<sup>1, 2</sup>

H. L. EASTERBROOKS, P. HELLER,<sup>3</sup> W. N. PLASTRIDGE AND E. L. JUNGHERR

*Storrs (Connecticut) Agricultural Experiment Station, Department of Animal Diseases,  
University of Connecticut, Storrs*

In 1949 Easterbrooks *et al.* (1) reported that the addition of 100 units ( $\mu\text{g.}$ ) of streptomycin sulfate per milliliter of diluted bull semen increased fertility significantly on the basis of a split sample study when used in the routine operation of the Connecticut Artificial Breeding Association. Mixner (3), reporting simultaneously showed neither significant increase nor decrease when streptomycin calcium chloride complex and penicillin were both added at rates of 1,000 units per milliliter. One source of variation between these studies was that different compounds of streptomycin were used.

The purpose of the present investigation was to test dihydro (DH) streptomycin sulfate and streptomycin calcium chloride complex (CCC), for evidence of incompatibility with semen diluent buffers, as well as for their comparative effectiveness in increasing fertility rates.

## EXPERIMENTAL

The problem was considered from two aspects. In the first phase of the study graduated concentrations of the two compounds varying from 100 to 1,000 units per milliliter in final dilution were added to phosphate and citrate buffers of various concentrations above, below and including those used routinely by artificial breeding organizations. After standing a few moments, a visible precipitate, calcium phosphate,<sup>4</sup> appeared in all tubes containing phosphate buffers to which streptomycin CCC had been added in excess of 100 units per milliliter. Because of the precipitation of these major components, the use of streptomycin CCC in phosphate containing diluents is considered contraindicated by the writers. No visible precipitation occurred at any level of either compound in any concentration of the citrate buffers.

Laboratory tests with the two compounds in citrate buffers revealed no measurable variation in respect to bactericidal activity or effect upon sperm livability.

The second phase of the work consisted of subjecting 14 ejaculates used by the Association over a period of 7 consecutive weekends in October and November, 1949, to a split sample study involving the two drugs at levels of 500 units per milliliter of diluted semen. This level was chosen in contrast to that previously

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<sup>3</sup> Manager, Connecticut Artificial Breeding Association.

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reported (1) because unpublished data indicate a level between 300 and 900 units to be optimum for increasing fertility; also 500 units per milliliter will destroy *Vibrio fetus* organisms which might be present in semen from infected bulls (4). The diluent used was composed of a 2.9 per cent sodium citrate and 0.6 per cent sulfanilamide in sterile distilled water buffer plus an equal volume of egg yolk. Comparisons were based on 60- to 90-day non returns (N.R.) to first service percentages. Five hundred eighty cows were inseminated with diluted semen containing DH streptomycin and 586 with diluted semen containing streptomycin CCC. First services only were used in compiling the data. The N.R. per cent for the DH streptomycin-treated semen was 71.6 as compared to 68.4 for the semen containing streptomycin CCC. The actual difference was 3.2 N.R. per cent and by a weighted analysis 2.3 N.R. per cent. These figures should be considered as precedent to those published by Easterbrooks *et al.* (2) in an abstract at an earlier date. No significance could be found associated with these data by statistical treatment; however, the data do suggest that DH streptomycin may be the drug of choice for addition to citrate containing diluents.

#### SUMMARY

Five hundred and eighty cows were inseminated with diluted semen containing 500 units of dihydro streptomycin sulfate per milliliter and 586 cows with split portions of the same semen containing 500 units of streptomycin calcium chloride complex. No statistical significance was associated with the 3.2 per cent non-returns increase for the dihydro streptomycin sulfate-treated group.

Streptomycin calcium chloride complex was found to be incompatible with phosphate buffers.

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## RESAZURIN REDUCING TIME AS AN INDICATOR OF BOVINE SEMEN FERTILIZING CAPACITY<sup>1</sup>

R. E. ERB AND M. H. EHLERS<sup>2</sup>

*Department of Dairy Husbandry, State College of Washington, Pullman*

Resazurin is a chemical indicator which, during its reduction, proceeds through a series of color changes. Resazurin is blue in milk or in water solution and reduces to resorufin which is pink in color. Resorufin further reduces to hydroresorufin, a colorless compound. The change to resorufin is irreversible, while the reduction to hydroresorufin is reversible (13). Resazurin has been extensively investigated for use as a rapid indicator of milk quality (2, 15). Its use now has gained considerable popularity because of its rapid reducing time, and it appears more versatile than the older methylene blue test as an indicator of milk quality. Methylene blue also has been used as an indicator of semen quality. This test was developed by Beck and Salisbury (1) and has been found to be quite highly correlated with initial motility and concentration. The basis of the test (1, 14) consists in determining time in minutes for semen diluted at a standard rate with yolk-citrate to reduce a 1 to 40,000 concentration of methylene blue.

The purpose of this paper is to report comparisons of the resazurin reduction time of bull semen to non-return rates, methylene blue reduction time, initial motility, concentration and survival at 3.3 and 45° C.

### EXPERIMENTAL

Semen samples were collected with the artificial vagina from eight young bulls at the State College of Washington and from 37 bulls regularly used for artificial breeding at the Northwest and Evergreen Co-op Breeders bull studs. Preliminary studies were started in January, 1948. Resazurin test solutions were prepared in distilled water at the rate of 11 mg. of the dye to 200 ml. The resazurin was procured in tablet form from the National Aniline Division of the Allied Dye Corp. Fresh solutions were made up once monthly and were stored

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in the refrigerator at 3.3° C. in brown glass bottles. No evidence of destruction could be detected up to 1 mo. in storage. The technique of the test was essentially the same as that described by Beck and Salisbury (1) for determination of methylene blue reduction time for semen, with the exception that the semen was undiluted. One-tenth ml. of the test solution was pipetted directly into a small culture tube containing 0.2 ml. of fresh undiluted semen, in a water bath at 45° C. The test sample was rotated in the bath to insure mixing and then layered with mineral oil. The pink endpoint was clear and complete. The white endpoint was adjudged as that time when 85 per cent of the column of semen was white. Methylene blue reduction time was determined in exactly the same manner, using 0.1 ml. of a 1:40,000 concentration of methylene blue and 0.2 ml. of undiluted semen.

Initially, resazurin reduction time was compared with such determinations as initial motility, concentration, ascorbic acid content and survival at 3.3 and 45° C. Initial motility in these studies was scored from 0 to 10, with 10 representing the maximum motility. Concentration was determined with the hemocytometer and ascorbic acid by the method of Roe and Keuther (9). In order to compare this test with fertilizing capacity, cooperative experiments were conducted with the two breeding associations located in Washington. Non-return rates (60 to 90 days) for all first and second services were determined on each sample by bulls, by local units and by age of semen at the time of use. Records of initial motility and concentration were kept. Resazurin and methylene blue reduction times were recorded on 507 semen samples unincubated prior to the tests and on 323 semen samples incubated for 30 min. at 45° C. before making the tests. Semen samples used for breeding 20 or more cows (first and second services) were used for statistical analyses, as suggested by Erb *et al.* (4). Non-return rates were expressed in per cent and converted to angles for final analysis. Statistical analyses were according to Snedecor (10).

#### RESULTS

Comparisons of resazurin reduction time to whole semen ascorbic acid content survival at 3.3 and 45° C. were made on 94 semen samples collected from three bulls between 1 and 2 yr. of age during the first half of 1948. The samples used for comparison ranged from 1 to 10 in initial motility. The results of this phase of the study are shown in table 1. The ascorbic acid content of 94 samples of whole semen average 8.0 mg. per cent as compared with 8.8 mg. per cent for the seminal plasma. For the purposes of this study, it was felt that values for whole semen were more applicable. Thirty semen samples which reduced resazurin to pink in 1 to 5 min. averaged  $8.7 \pm 0.51$  mg. per cent, as compared with  $6.7 \pm 0.68$  mg. per cent ascorbic acid for 12 samples requiring 21 to 60 min. for reduction to pink. The five samples which did not completely reduce to pink in 1 hr. averaged slightly higher ( $7.1 \pm 1.35$ ), but the high standard error reflects the need for more samples in this range. Thirty-six of the 94 samples studied reduced resazurin to the white endpoint in 30 min. or less. The average ascorbic acid content of these samples was  $8.5 \pm 0.41$  mg. per cent, compared

TABLE 1  
Resazurin compared with whole semen ascorbic acid content and survival time at 3.3 and 45° C.

Pink endpoint						White endpoint					
Reduction time interval	No. Samples	Av. time within intervals	Ascorbic Acid	Survival at:		Reduction time interval	No. Samples	Av. time within interval	Ascorbic Acid	Survival at:	
				3 °C.	45° C.					3 °C.	45° C.
(min.)		(min.)	(mg. %)	(d.)	(min.)			(min.)	(mg. %)	(d.)	(min.)
1-5	30	3 6	8 7±0 51	15 2±1 4	81 8±5 7	0-30	36	18 9	8 5±0 41	14 1±1 0	89 3±7 7
6-10	21	7 5	7 7±0 50	11 2±1 4	113 8±14 3	31-60	18	40 4	9 0±0 58	12 9±2 0	106 5±14 8
11-15	20	13 2	8 3±0 36	10 5±1 4	113 2±14 6	Partial					
16-20	6	18 0	8 7±1.01	5 6±1 8	84 8±33 8	in 60	23	...	7 4±0 38	10 1±1 9	102 0±13 4
21-60	12	32 5	6 7±0 68	8 8±3 4	46 9±15 5	>60	17	...	6 9±0 68	4 6±1 3	41 9±11 2
>60	5	...	7 1±1 35	3 6±2 4	25 5±8 2			...			
Total	94	....	8 0±0 25	11 4±0 8	87 6±6 1	Total	94	...	8 0±0 25	11 4±0 8	87 6±6 1



with  $6.9 \pm 0.68$  mg. per cent for 17 samples showing no reduction to white in 60 min. By analysis of variance (93 d.f.) the relationship of ascorbic acid level to resazurin reduction to pink was not significant. The same comparison with the white endpoint approached significance at the 5 per cent level. From these limited data representing all levels of semen quality, it appears the quantity of ascorbic acid in semen does not materially affect the resazurin reduction test.

Survival time under storage (undiluted semen) at  $3.3^{\circ}$  C. and under incubation at  $45^{\circ}$  C. varied inversely with reducing times for both the pink and white endpoints. Survival time in each case was measured to zero motility. The variation between the means for the two measures of survival for the respective time intervals for resazurin reduction shown in table 1 were highly significant.

TABLE 2  
*Comparison of initial motility with resazurin reduction time*

Initial motility rating	Pink				White			
	No. samples	Reduced in 1 hr.	Av. re- duction time <sup>a</sup>	Range <sup>b</sup>	No. samples	Reduced in 1 hr.	Av. re- duction time <sup>a</sup>	Range <sup>b</sup>
		(%)	(min.)	(min.)		(%)	(min.)	(min.)
0	6	0.0	—	—	6	0.0	—	—
1	26	76.9	40.3	12-60	27	33.3	40.0	14-54
2	18	77.8	24.6	5-60	19	26.4	27.0	9-59
3	29	79.3	22.3	3-60	27	33.3	39.3	5-60
4	13	84.6	15.5	4-60	13	30.8	25.5	19-60
5	31	96.8	18.3	2-60	31	48.4	34.1	17-60
6	39	100.0	7.2	1-22	39	87.2	32.9	7-60
7	89	100.0	3.9	1-9	89	92.1	22.3	7-60
8	92	100.0	2.9	1-16	92	97.8	20.1	4-60
9	18	100.0	2.5	1-9	98	100.0	16.7	5-30
10	123	100.0	1.2	1-3	123	100.0	9.3	3-30

<sup>a</sup> Av. for samples that reduced in 1 hr. or less.

<sup>b</sup> Range for samples that reduced in 1 hr. or less.

Pink and white reduction endpoints were compared on 564 semen samples with respect to initial motility. The results (table 2) reveal that the six samples rating zero motility also failed to reduce resazurin to purple, which is an intermediate color in the reduction to pink. Some samples failed to reduce to pink in 1 hr. until initial motility exceeded a rating of 5. The average reduction time to pink was more than twice as short with a motility of 6 as compared with a motility of 5. While some samples with motility of 6 to 8 failed to reduce completely to white in 1 hr., the breaking point appears to be between 5 and 6. Only 48.4 per cent of the samples rating 5 reduced to white, as compared with 87.2 per cent for samples rating 6. The 223 samples given initial motility ratings of 9 or 10 all reduced resazurin to white in less than 1 hr. Since it generally is agreed that samples rating below 5 are undesirable for routine use in artificial insemination, correlations were determined for only those samples, used by Northwest Co-op. Breeders, rating above 5. The correlation was  $-0.459$  for 376 samples for the Guernsey, Jersey and Holstein breeds, which showed

individual breed correlations of  $-0.475$ ,  $-0.529$  and  $-0.460$ , respectively. Similar correlations for white resazurin for all breeds was  $-0.232$  and was  $-0.232$ ,  $-0.473$  and  $-0.097$  for the Guernsey, Jersey and Holstein breeds, respectively.

Concentration also was reflected in the resazurin reduction times, as shown in table 3. Samples reducing to pink in 5 min. or less and to white in 1 hr. or less, averaged 1 million or more sperm per mm.<sup>3</sup>. The correlation of pink resazurin and concentration for samples used by Northwest Co-op. Breeders was  $-0.399$  for all breeds and was  $-0.391$ ,  $-0.589$  and  $-0.333$  for the Guernsey, Jersey and Holstein breeds, respectively. Similar correlations for white resazurin was  $-0.267$  for all breeds and  $-0.220$ ,  $-0.535$  and  $-0.181$  for the Guernsey, Jersey and Holstein breeds, respectively. The correlation of concentration to motility for these same samples also was high, being 0.478 for all breeds and 0.536, 0.554 and 0.288 for the Guernsey, Jersey and Holstein breeds, respectively. The spermatozoa were

TABLE 3

*Comparison of concentration of spermatozoa per mm.<sup>3</sup> with resazurin reduction time for all semen samples showing an initial motility rating of one or higher*

Pink				White			
Reducing time	No. samples	Av. concentration	Range	Reducing time	No. samples	Av. concentration	Range
(min.)		(thousands /mm <sup>3</sup> )	(thousands /mm <sup>3</sup> )	(min.)		(thousands /mm <sup>3</sup> )	(thousands /mm <sup>3</sup> )
1	125	1,850	920-3,540	3 or less	8	2,430	1,920-3,170
2	136	1,340	690-2,280	4-6	49	1,910	690-3,540
3	76	1,100	320-1,760	7-9	66	1,580	630-2,920
4	47	1,050	510-2,200	10-12	76	1,370	820-2,300
5	21	1,090	730-2,240	13-15	72	1,280	500-2,740
6	16	990	590-1,880	16-18	39	1,240	560-2,540
7	7	890	560-1,290	19-21	37	1,070	420-2,070
8	11	990	420-2,040	22-24	19	1,140	510-2,280
9-10	11	900	500-2,370	25-27	10	1,010	650-1,880
11-15	30	910	260-1,760	28-30	21	1,190	550-2,040
16-60	55	740	200-1,760	31-60	57	1,000	500-2,370
>60	23	490	100-940	>60	104	750	200-2,060

centrifuged from 12 samples with high initial motility and high concentration. No resazurin reduction was observed in the seminal plasma of the samples during 1 hr. of incubation. Hence, for detectable reduction of resazurin, motile spermatozoa are required.

Two separate experiments were conducted to test the fertilizing prediction value of the resazurin test. The first involved 34 bulls and 507 semen samples from Northwest and Evergreen Co-op. Breeders. In this trial, resazurin and methylene blue reduction times were determined as outlined previously. The second trial, involving 20 bulls and 304 semen samples from Northwest Co-op. Breeders, varied from the first only in that the undiluted semen was incubated for 30 min. at 45° C. before adding resazurin and methylene blue to the test vials to determine the reduction time. This was undertaken in an effort to speed up reduction of resazurin to white, since preliminary analyses of the data from the first trial indicated no correlation of the white endpoint to non-return rates.

Average reduction time of resazurin to pink was lowered from 2.28 min. per sample to 1.56 min., and average time to reduce to white was lowered from 16.7 to 5.9 min. Methylene blue reduction was slowed by incubating before making the test on undiluted semen. The average reduction time for unincubated semen was 29.4 min., as compared with 84.0 min. for incubated semen. The reason for this is not immediately apparent but possibly indicates that resazurin and methylene blue do not measure the same reducing properties of semen. The correlations between the reduction tests were as follows: (a) Unincubated semen; methylene blue compared with pink and white resazurin was 0.430 and 0.382, respectively, and pink compared with white resazurin was 0.563. (b) Incubated semen; methylene blue compared with pink and white resazurin was 0.499 and 0.596, respectively, and pink compared with white resazurin was 0.741. Even though the average methylene blue reduction time was increased by incubation and the reduction of resazurin to pink and white was decreased, the correlations were higher than for unincubated semen.

Comparisons of resazurin reduction time to non-return rates are shown in tables 4 and 5. Non-return rates are reported by two methods in these tables. The left-hand column in each section of each table shows the non-return rate based on total first and second services on all semen samples studied. The corresponding right-hand column shows the average non-return rate converted from per cent to angles and averaged. These averages then were reconverted to per cent, as shown in the right hand column of each section of each table. This latter procedure was necessary in order to determine correlations and make tests of significance and will be referred to in the discussion. In general, the non-return rates were slightly higher when averaging non-return rates on a per sample basis.

As shown in table 4, 125 unincubated semen samples which were used for breeding 20 or more cows and which reduced resazurin to pink within 1 min. were significantly superior, as shown by the analysis of variance (370 d.f.). The difference between non-return rates for semen reducing resazurin to pink in 1 min. or less, compared with 4 min. or more for all breeds, was 8.0 per cent. This mean difference was highly significant. For incubated semen, 182 samples reduced resazurin to pink in 1 min. or less and were 8.7 per cent higher than incubated semen requiring 3 min. or more. This mean difference also was highly significant (303 d.f.).

Reduction time of resazurin to white on unincubated semen was of no value for estimating fertilizing capacity. When the semen was incubated for 30 min. before making the test (table 5), 43 semen samples reducing resazurin to white in 3 min. showed an average non-return rate of 67.6 per cent, as compared with 66.2 per cent for 40 samples requiring 1 to 2 min. for reduction to white. The latter is 6.0 per cent higher than the average non-return rate of 60.2 per cent for 24 samples requiring more than 10 min. for reduction. The differences between the means for 1, 2 and 3 min., as compared with over 10 min., were highly significant. The variance for between times likewise was highly significant (303 d.f.).

TABLE 4  
Comparison of resazurin reduction (pink endpoint) time of undiluted semen and fertilizing capacity

Reduction time (min.)	Unincubated				Incubated			
	No. samples	No. services	Non-returns (%)	Samples—available for statistical analysis (no.)	No. samples	No. services	Non-returns (%)	Samples—available for statistical analysis (no.)
<b>Breed—Guernsey</b>								
1	65	2429	63.8	50	90	5126	65.6	90
2	76	2676	59.5	76	40	2206	63.6	40
3	42	1118	56.9	28	4	248	57.4	4
4	21	794	60.0	19	2*	40	66.7	2
>4	10	435	53.8	10				
Total	214	7452	60.2	183	136	7620	64.8	136
<b>Breed—Jersey</b>								
1	72	1699	59.2	45	52	1869	61.4	50
2	34	867	57.7	25	32	963	58.3	27
3	29	653	62.3	18	4	79	63.3	1
4	8	160	53.1	4	7*	177	56.5	5
>4	9	131	62.6	2				
Total	152	3510	59.3	94	95	3088	60.2	83
<b>Breed—Holstein</b>								
1	46	1253	66.3	30	46	1526	65.5	42
2	39	970	64.0	35	33	1224	63.8	31
3	32	614	64.5	15	9	377	66.6	9
4	12	232	58.6	7	4*	110	58.2	3
>4	12	322	57.4	7				
Total	141	3391	64.0	94	92	3237	64.7	85
<b>All Breeds</b>								
1	188	5381	63.0	125	188	8521	64.6	182
2	144	4513	60.1	136	105	4393	62.5	98
3	103	2385	60.3	61	17	705	63.0	14
4	41	1186	58.8	30	13*	326	58.3	10
>4	31	888	56.4	19				
Total	507	14353	60.9	371	323	13945	63.7	304

\* 4 min. or longer





The correlations for resazurin and methylene blue reduction times for unincubated and incubated semen and non-return rates by breeds are shown in table 6. The correlations for initial motility and concentration to non-return rates also are shown for each trial. Time required to reduce unincubated semen to pink showed a highly significant correlation of  $-0.141$  to fertility. Likewise, in the first trial, resazurin reduction to white, methylene blue reduction time, initial motility and concentration showed no significant correlations with non-return rates. When the semen was incubated, pink resazurin reduced quite rapidly, but did not lose sensitivity, since the correlation of  $-0.151$  to non-return rate was highly significant.

Average time to reduce resazurin to white was approximately three times faster for incubated semen. The correlation for the white endpoint of  $-0.169$  to fertility was highly significant. Likewise, during this trial, initial motility

TABLE 6  
*Correlation Summary by breeds*

Non-return rate compared to:	Guernsey		Jerseys		Holstein		All Breeds	
	No. Samples	r	No. Samples	r	No. Samples	r	No. Samples	r
<i>Reduction times on unincubated semen</i>								
Pink resazurin	183	$-0.291^{**}$	94	$-0.022$	94	$-0.210^{*}$	371	$-0.141^{**}$
White resazurin	183	$-0.032$	94	$+0.087$	94	$-0.186$	371	$-0.031$
Methylene blue	150	$-0.073$	65	$-0.039$	73	$-0.186$	285	$-0.046$
Initial motility	183	$+0.080$	94	$+0.006$	94	$+0.006$	371	$+0.022$
Concentration	183	$+0.073$	94	$-0.098$	94	$-0.194$	371	$0.000$
<i>Reduction times on incubated semen</i>								
Pink resazurin	136	$-0.136$	83	$-0.217^{*}$	85	$-0.204$	304	$-0.151^{**}$
White resazurin	316	$-0.201^{*}$	83	$-0.176$	85	$-0.115$	304	$-0.169^{**}$
Methylene blue	136	$+0.011$	83	$-0.158$	85	$-0.085$	304	$-0.090$
Initial motility	136	$+0.228^{**}$	83	$+0.061$	85	$+0.271^{*}$	304	$+0.207^{**}$
Concentration	136	$+0.185^{*}$	83	$+0.060$	85	$+0.155$	304	$+0.163^{**}$

\* = significant

\*\* = highly significant

and concentration were highly-significantly correlated with non-return rate. When the two trials were combined, giving a total of 675 semen samples used on 20 or more cows, correlations of 0.076 and 0.052 for initial motility and concentration, respectively, to non-return rate were observed. This suggests a possible seasonal difference, since semen used for the first trial was collected from July to March and semen for the second trial was collected from March to August. The data also were analyzed by age of semen. Since too few samples were used to breed 20 or more cows during any one 24-hr. period, the correlations were low and erratic. Summarizing on the basis of totals for first and second services revealed that the quality tests herein reported on had no additional predictive value with respect to age of semen at the time of insemination.

#### DISCUSSION

Resazurin reduction time to pink and white has shown some promise in this experiment as an indicator of semen quality. Ascorbic acid content of the whole

semen did not appear to affect the resazurin reduction time of 94 samples of semen. The average for these 94 samples was 8.0 mg. per cent, which is higher than a range up to 8 mg. per cent reported by Phillips *et al.* (8). VanDemark *et al.* (13) failed to associate methylene blue reduction with ascorbic acid content of semen, although Beck and Salisbury (1) had felt earlier that the two were correlated. These latter authors also reported that the methylene blue test also was largely dependent on concentration and rate of motility. In this respect, resazurin and methylene blue reduction times give relatively similar results. Beck and Salisbury (1) reported correlations of  $-0.6532$  and  $-0.6577$  for methylene blue compared with concentration and initial motility, respectively. In this experiment, the correlations of concentration to pink and white resazurin and methylene blue reduction times were  $0.399$ ,  $-0.267$  and  $-0.449$ , respectively, and for initial motility  $-0.459$ ,  $-0.232$  and  $-0.493$ , respectively. The correlation of initial motility to concentration was  $0.478$ .

The time required to reduce resazurin to pink and white was inversely related to survival time at  $3.3$  and  $45^{\circ}$  C. Swanson and Herman (11), using grouped data, observed a highly significant correlation between conception rate and survival under storage. Madden *et al.* (7) could demonstrate no significant difference between longevity under cold shock conditions and conception rate. Erb and Shaw (5) could demonstrate no correlation between motility after 30 min. incubation at  $45^{\circ}$  C. and non-return rate.

Resazurin reduction time to pink gave a highly significant correlation with non-return rate on 371 semen samples. This correlation of  $-0.141$ , plus the close relationship to survival under storage, initial motility and concentration, makes this particular test appear promising. A recent experiment (3) has shown that when concentration of semen was adjusted to approximately 750,000 sperm mm.<sup>3</sup>, the correlation coefficient between resazurin reduction to pink and non-return rate was  $-0.517$  on 72 samples. This technique increased the selectivity of the pink endpoint by removing some of the effects of variable concentration. Efforts to set up a series of standards utilizing initial motility, concentration and resazurin reduction failed to improve non-return rates in the sub-classes of superior semen quality over the differentiation observed by using resazurin reduction time to pink as the only criterion.

#### SUMMARY

Resazurin was tested as a possible indicator of fertilizing capacity. The test for reduction time was made by using 11 mg. of resazurin dye in 200 ml. of distilled water. One-tenth ml. of this solution was added to 0.2 ml. of undiluted semen and incubated at  $45^{\circ}$  C. Time required for reduction to pink, the first endpoint, and reduction further to white was recorded for 924 semen samples from 45 bulls representing the Guernsey, Jersey and Holstein breeds. The relationship of the pink and white endpoints to survival at  $3.3$  and  $45^{\circ}$  C. (94 semen samples), initial motility (564 semen samples), concentration (558 semen samples) and methylene blue reduction time (376 semen samples) was high. The time required to reduce to pink in unincubated semen showed a highly sig-



nificant correlation of  $-0.141$  to non-return rate on 371 semen samples which were used for 20 or more first and second services. The white endpoint showed a slightly higher correlation of  $-0.169$ , as compared with  $-0.151$  for the pink endpoint when the semen was incubated for 30 min. at  $45^{\circ}$  C. before making the test.

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## THE DETERMINATION OF LINOLEIC ACID IN MILK FAT

P. S. SCHAFFER<sup>1</sup> AND GEORGE E. HOLM

*Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A., Washington, D. C.*

Until recently, when the photoelectric spectrophotometer came into general use as an aid in fat analysis, it has been practically impossible to obtain accurate data on the amounts of the unsaturated acids in fats. Most of the naturally occurring fats do not possess chromophores but possess structures which may be altered by chemical means to produce groups which absorb radiant energy. For example, linoleic acid possesses a diene grouping and when treated with alkali forms an isomer containing a conjugated double bond, which structure causes an absorption of light in a region of the ultraviolet spectrum. The intensity of the absorption may be used as a basis for measuring the amount of linoleic acid present in a fat.

Mitchell *et al.* (1) described a procedure for the quantitative estimation of linoleic and linolenic acid content of various fats and oils. By using pure linoleic and linolenic acid, they obtained reference standards at the points of maximum absorption, namely 234  $m\mu$  and 268  $m\mu$ , respectively, which may be used in the determination of these acids in mixtures of other fat acids. Beadle and Kraybill (2) later published reference values for linoleic acid and linolenic acid which they obtained with a Beckman spectrophotometer. Riemenschneider *et al.* (3), using an adsorption fractionation technique, were able to isolate methyl linoleate which gave a higher spectrophotometric absorption coefficient than previously reported. Brice and Swain (4), by using alkaline glycerol as an isomerizing medium, described a method for simultaneous spectrophotometric determination of non-conjugated and conjugated diene, triene and tetraene fat acid constituents of vegetable oils, animal fats, their soaps and purified fat acid preparations. Stainsby (5) describes a method for the determination of linoleic acid by oxidation of the fat in acetone, followed by titration of the acidic glycerides after removal of the steam-volatile acid products.

The literature dealing with the determination of linoleic acid in milk fat is limited. Eckstein (6), by using the lead-salt method of separating the saturated from the unsaturated fat acids in milk fat, was able to obtain only about 0.2 per cent linoleic acid and about 0.1 per cent linolenic acid. Hilditch *et al.* (7), in an examination of the glycerides of milk fat which had been separated by low temperature crystallization from acetone, reported the linoleic acid content to be about 5.5 per cent. Later, Hilditch and Jasperson (8), with the aid of a quartz spectrograph and using a concentrate of the more unsaturated acids of cow milk fat, prepared by lithium salt separation, reported the presence of a total of 2 per cent non-conjugated and 2 per cent conjugated octadecadienoic acid. They also reported traces of conjugated and non-conjugated octadecatrienoic acids.

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<sup>1</sup> Now with the Bureau of Agricultural and Industrial Chemistry.

White and Brown (9), in a study of the tetrabromide method of estimating linoleic acid in fat acid mixtures, were able to definitely identify linoleic acid in butterfat by actual isolation of the tetrabromide.

With the aid of a Beckman spectrophotometer and the adoption of some of the existing procedures, the amount of linoleic acid present in milk fat has been determined.

#### EXPERIMENTAL

*Preparation of isomerizing reagent.* The method used for the preparation of the ethylene glycol-KOH isomerizing reagent was essentially as described in specific detail by O'Connor *et al.* (10). The reagent was prepared in an atmosphere of nitrogen, was colorless and permitted the use of a larger sample of the milk fat acids in the procedure. The reagent consists essentially of a solution containing 7.5 g. of 85 per cent. KOH per 100 ml. of ethylene glycol.

*Preparation of milk fat acids.* Samples of butter were converted to butteroil by heating the butter at 60° C. until melted. Prolonged heating was avoided in order to eliminate oxidation. The melted fat was separated from the curd and water by centrifuging in glass bottles. Approximately 100 g. of the butteroil then were saponified as prescribed by Jamieson (11). The potassium soaps were converted to the free fat acids by means of HCl and the free fat acids were isolated by extracting with peroxide-free ethyl ether. The last traces of ether were removed under vacuum.

*Isomerization of milk fat and milk fat acids.* Samples containing approximately 0.1 g. fat or fat acids were weighed out in small glass vials and were added to 10 ml. of the ethylene glycol-KOH solution in pyrex test tubes that were being held in a constant temperature bath at 180° C. as prescribed by O'Connor (10). After 25 min. the tubes were removed from the bath and cooled quickly in a cold water bath. The contents of the tube then were transferred quantitatively to a 100-ml. volumetric flask, using 95 per cent alcohol purified by distillation over Zn and KOH, to wash out the tubes. The solutions usually require further dilution before they can be used in the spectrophotometer. A sample of the isomerizing reagent was treated similarly and was used as the reference material in the spectrophotometer.

*Spectrophotometric measurements.* A Beckman DU photoelectric spectrophotometer employing a hydrogen lamp was used to measure the optical densities. The solutions, after being filtered through sintered glass funnels just before being used, were placed in 1-cm. cells for reading of the optical densities. Density readings were made in the range 224–270 m $\mu$ . The specific absorption coefficient ( $a$ ) was calculated for the various wavelengths, using the equation  $\text{Specific } a = \frac{E}{cl}$ , where  $a$  = specific absorption coefficient,  $E$  is the optical density (obtained as a direct reading on the spectrophotometer),  $c$  is the concentration of solute in grams per 1000 ml. and  $l$  is the length in centimeters of solution through which the radiation passes.

## RESULTS

*With milk fat acids.* Figure 1 shows a typical absorption curve for isomerized milk fat acids. At  $234\text{ m}\mu$ , the intensity of absorption is due to diene and triene conjugation which results from the isomerization of the linoleic and apparent linolenic acids present. The absorption at  $268\text{ m}\mu$  is due to a triene conjugation. This absorption is a measure of the apparent linolenic acid present in the milk fat acids. It has been shown by others (10, 12) that small but interfering absorption takes place at  $268\text{ m}\mu$  even though the presence of linolenic

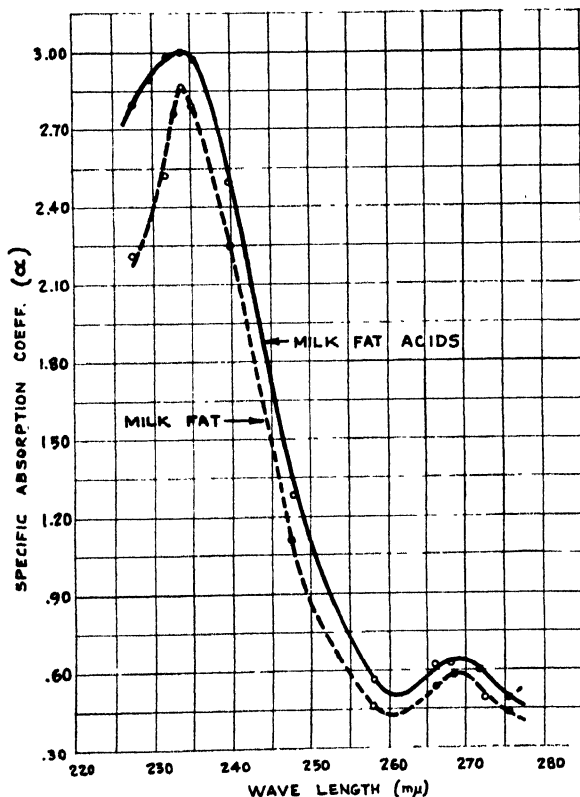


FIG. 1.—The specific absorption coefficients of isomerized milk fat and milk fat acids in alcohol solutions at different wavelengths of light.

acid can not be proved by actual isolation of a hexabromostearic acid. However, in the case of the milk fat acids, although no attempt was made to actually prove the presence of linolenic acid by the isolation of hexabromostearic acid, the absorption at  $268\text{ m}\mu$  is greater than is warranted by an interfering substance due to isomerization of the linoleic acid. Therefore, it has been called the apparent linolenic acid present in the fat. Since both diene and triene conjugation absorb radiant energy at  $234\text{ m}\mu$ , it is necessary to make a correction at  $234\text{ m}\mu$ , in cal-

culating the amount of linoleic present, for the absorption due to diene conjugation resulting from the apparent linolenic acid present in the fat. The equations for calculating the amount of linolenic and linoleic acids are as follows:

$$\text{per cent linolenic acid} = Y = \frac{a (268 \text{ m}\mu) \times 100}{53.2}$$

$$\text{per cent linoleic acid} = \frac{a (234 \text{ m}\mu) - \left( \frac{Y}{100} \times 60.9 \right)}{86.0} \times 100$$

where 53.2 is the specific absorption coefficient of pure linolenic acid at 268 m $\mu$ , 60.9 is the specific absorption coefficient of linolenic acid at 234 m $\mu$  and 86.0 is the specific absorption coefficient of pure linoleic acid at 234 m $\mu$ .

Absorption values for unisomerized milk fat and milk fat acids dissolved in iso-octane, using iso-octane as a blank, (4) increased very slightly only in the region of wavelengths of approximately 269 m $\mu$ , indicating that practically no conjugated systems existed in the original fat.

Table 1 shows the amount of linoleic acid and apparent linolenic acid calculated to be present in some of the samples analyzed.

TABLE 1

Milk fat source	Linoleic acid after correction for triene conjugation	Octadecatrienoic acid calculated as linolenic acid
	(%)	(%)
1. Winter butter (past. cream)	2.11	1.29
2. Spring butter (unpast. cream)	2.17	1.05
3. Same (past. cream)	2.11	1.20
4. Whey cream butter (Swiss)	2.31	1.11
5. Summer butter (past. cream)	2.42	1.09

In order to test the accuracy with which the spectrophotometric observations may be made, pure linoleic acid was used to fortify some of the butter fat acid samples, using up to a maximum of 3 per cent linoleic acid based on the weight of the milk fat acid sample. An identical sample of milk fat acids was used in the blank. The maximum deviation from complete recovery of the linoleic acid at 234 m $\mu$  was  $\pm 0.8$  per cent for a solution containing 1 per cent added linoleic acid and  $\pm 0.5$  per cent of the amount present for a solution containing 3 per cent added linoleic acid.

*With milk fat.* Figure 1 also shows the absorption data obtained with isomerized milk fat. Table 2 gives the values obtained for the percentage of linoleic acid in milk fats, when the milk fat or its fat acids are used in the determination. Sample 1 was a fresh butterfat, while sample 2 had been prepared and stored at a temperature of 40° C. for 1 yr. before isomerization and optical density readings were made.

#### SUMMARY

A spectrophotometric method is described for the determination of linoleic acid in milk fat. Values are given for the linoleic acid and apparent linolenic

acid content of samples of milk fat obtained from whey and from summer and winter milk.

TABLE 2

Milk fat source	Linoleic acid	Octadecatrienoic acid calculated as linolenic acid
	(%)	(%)
1. Summer 1949 milk fat	2.62	1.02
Summer 1949 milk fat acids	2.63	1.17
2. Summer 1948 milk fat	2.71	0.77
Summer 1948 milk fat acids	2.64	0.81

## ACKNOWLEDGMENT

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## AN ALL-ROUGHAGE RATION FOR BULLS<sup>1, 2</sup>

G. V. QUICKE AND P. H. PHILLIPS

*Department of Biochemistry, University of Wisconsin, Madison*

AND

W. H. DREHER

*Badger Breeders Cooperative, Shawano, Wisconsin*

The rapid development of large scale artificial breeding programs in areas devoted largely to the raising of dairy cattle has resulted in the establishment of breeding rings dependent upon the maintenance of large bull studs. It is not surprising, therefore, that the feeding of bulls has received increased attention during the last few years.

The cost of feed is an important contributory factor to the over-all cost of maintaining such large bull studs, and any means whereby these costs can be reduced should prove of considerable advantage. The relatively high cost of grain-concentrate mixtures suggests a more economic utilization of such mixtures as an important step in this direction. One measure advocated by Reid, *et al.* (8, 9) is the use of simple, rather than complex, concentrate mixtures.

Although the inclusion of grain in the ration generally has been accepted as necessary in the feeding of dairy bulls used for breeding purposes, there is little experimental evidence either to support or refute this practice. Since the bull is a ruminant, it should be able to make the best use of roughage feeds, and an investigation into the possibility of feeding a ration devoid of any concentrate mixture appeared justifiable.

The roughages used to make up such a ration necessarily must be of a high quality and the over-all digestible crude protein and TDN content of the ration should be maintained at the levels known to give satisfactory results. It was realized that in order to develop such a ration it would be necessary to utilize large amounts of silage. It generally is believed that too much silage results in "excess middle" and a consequent falling off in the libido of bulls. There are again no pertinent data in support of this idea. It was hoped, therefore, that the use of a ration consisting of good hay and a high level of good quality silage would provide information on the latter subject.

### EXPERIMENTAL

*Selection of bulls.* Twenty bulls, 10 Holsteins and 10 Guernseys, were selected on the basis of their age, general thrift, vigor and condition, heart and paunch girth, semen quality and, where available, the past year's conception rate, in such

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a way that they could be divided into two equalized groups. Group I was designated the "control" group, while group II was the "experimental" group. The above data for the individual bulls, as well as the distribution of the paired bulls between the two groups, are presented in table 2.

Unfortunately, 4 mo. after the experiment was started three of the bulls (one experimental Holstein and two control Guernseys), due to circumstances beyond the control of the experiment, were sold for slaughter. Therefore, data pertaining to these bulls were discarded.

*Rations and management of bulls.* A ration consisting of 5 lb. of a concentrate mixture (ground corn and oats, wheat bran, linseed oil meal, soybean oil meal, bone meal, blood meal, tankage and mineral salt mix), 15 lb. of grass-legume silage and good quality mixed hay fed *ad libitum* per bull per day, had

TABLE 1

*Rations fed and calculated intake of T.D.N. and D.C.P. per bull per day, based on the intake of a 2000-lb. bull*

	lb. feed/100 lb. live weight	Total intake	T.D.N. <sup>a</sup>	D.C.P. <sup>a</sup>	N.R.
		(lb.)	(lb.)	(lb.)	(lb.)
<i>Control ration:</i>					
Grain mixture	0.25	5.0	3.9	0.7	
Silage (grass-legume)	0.75	15.0	3.0	0.45	
Hay (grass-legume) <sup>b</sup>	1.25	25.0	11.2	1.25	
Total			18.1	2.40	1:6.5
<i>Experimental ration:</i>					
Silage (grass-legume)	2.25	45.0	9.0	1.35	
Hay (grass-legume) <sup>a</sup>	1.25	25.0	11.2	1.25	
Total (grass-legume) <sup>a</sup>			20.2	2.60	1:6.7

<sup>a</sup> Calculated from Morrison's tables on the composition of feeds.

<sup>b</sup> Hay was fed *ad libitum*, the intake used in the above calculations being based on an estimate.

been used with excellent results since the inception of the stud in which the experiment was set up. This ration was used, therefore, as the control (lot I) against which an all-roughage ration (lot II) could be tested.

The hay consisted of alfalfa and brome grass, together with a small amount of red clover. The silage was made from these same three forage crops, 20 lb. of molasses being added per ton of silage. The two rations, together with data calculated from Morrison's tables (5), on their TDN, digestible crude protein content and nutritive ratios are presented in table 1.

The bulls used in the experiment were treated in the same way as the rest of the bulls in the stud, *i.e.*, exercise, time of feeding, semen collection (5-day intervals) etc. were continued in the same manner as had been done prior to the setting up of the experiment.

Observations such as volume, initial motility and density by microscope estimation of ejaculates were made routinely with each collection. The duration of motility in storage was determined from time to time. The semen was used in



TABLE 2  
*Representative data on various characteristics of typically paired bulls and the basis of pairing the bulls*  
 Holstein

Bull	Group allocated	Age at start of expt.	Heart girth		Paunch girth		Vigor, thrift and condition		Semen rating		Conception rate	
			Initial	Final	Initial	Final	Initial	Final	Pre-exptl. period	Exptl. period	Pre-exptl. period	Exptl. period
		(yr. mo.)	(in.)	(in.)	(in.)	(in.)					(%)	(%)
H 20	I	10-0	90.5	89	101.5	101	Ex	Fair	4	4	63.3	71.4
" 34	II	11-4	98	95.5	110.5	111	Good	Good	4	4	64.7	63.3
" 26	I	5-0	94.5	95.5	105	107.5	Ex	Ex	4	4	61.4	68.8
" 35	II	5-8	94	90	105	106.5	Ex	Good	4	4	61.8	65.1
" 37	I	1-8	75	88	87.5	101	Ex	Ex	4	4	66.5	70.9
" 38	II	1-9	77	85	93.5	104	Ex	Ex	4	4	69.8	71.4
Guernsey												
G 51	II	5-5	83	82.5	99	100	Ex	Ex	4	4	26.9	60.4
" 47	I	6-8	84.5	88.5	99.5	105	Ex	Ex	4	4	66.6	68.8
" 36	II	6-7	87	85	101	102	Ex	Ex	4	4	56.0	62.9
" 57	I	1-2	63.5	78	76.5	93.5	Ex	Ex	3-4	4	No rec	67.5
" 58	II	1-2	63.5	76	73	90	Ex	Ex	4	4	No rec	69.3

the field for artificial insemination and all pertinent records were kept by the breeding ring.

Chemical analyses were conducted on semen from all the bulls at the start and end of the experiment. At 4-wk. intervals during the course of the experiment, semen for chemical analysis was taken from one-fourth of the bulls in such a way that semen collection schedules of the breeding ring were undisturbed. The analyses carried out included ascorbic acid, total nitrogen, non-protein nitrogen and acid and alkaline phosphatase activity.

The ascorbic acid content of the semen was determined by means of a modification of the method of Mindlin and Butler (4), using a Fisher electrophotometer. These determinations were made immediately following collection of the semen.

Total nitrogen was determined by means of a semi-micro Kjeldahl method,  $\text{CuSO}_4$  being used as catalyst for the digestion.

Since it was desired to determine the level of non-protein nitrogen in whole semen, it was necessary to develop a method in which the non-protein nitrogen of the sperm cells would be liberated. Zittle and O'Dell (12) reported that the cell wall of the spermatozoon can be dissolved in the presence of an alkali and  $\text{Na}_2\text{S}$ , and this observation was utilized in developing the following method: Two ml. of semen were transferred to a 25-ml. volumetric flask, 2 ml. of a 0.2*M*  $\text{Na}_2\text{S}$  solution in 2*N*  $\text{NaOH}$  were added and the mixture was allowed to stand for 5 min. A clear, straw-colored viscous solution resulted. Two ml. 2*N*  $\text{HCl}$  were added while shaking the flask vigorously and a white precipitate started to form; 10 ml. of a 10 per cent trichloroacetic acid solution were added to complete the precipitation of the proteins. The flask was shaken vigorously and then allowed to stand for 15 to 30 min., the contents made up to volume with distilled water and the precipitate filtered off by means of a fluted Whatman no. 42 filter paper. A 5-ml. aliquot of the clear filtrate was used for the determination of nitrogen by the semi-micro Kjeldahl method. (After the filtration had stood for a short time, a fine white precipitate settled out. This was due to the liberation of free S from the  $\text{Na}_2\text{S}$  reagent.)

The estimation of phosphatase in semen was obtained with a modified method developed by Johnson (3). In the latter method, a 2-ml. aliquot is taken from the reaction mixture for testing purposes. The advantage of the small aliquot lies in the fact that it avoids the formation of a precipitate. A 0.1*M* ethylene diamine-citrate solution of the desired pH (5.0 for the acid- and 9.3 for the alkaline-phosphatase) was used as buffer.

The time of survival of the spermatozoa was determined in the semen samples that were taken for chemical analysis. A portion of the semen was diluted immediately after collection with egg yolk-phosphate diluent (6) to which penicillin had been added at a level of 100,000 units per 100 ml. diluent. A dilution rate of 1:20 was used and the diluted semen was stored in a refrigerator at 4° C. Motility was estimated every second day with the aid of a warm-stage microscope.

In order to obtain a measure of the fertility of the two groups of bulls, non-return data were assembled in the following manner: (a) 90-day non-return data from cows receiving first service or first service after calving, were utilized. A

cow was recorded as a 90-day non-return if she was not reported for a second insemination within 90 days from the date of the first service. (b) Only paired data were used, i.e., where a pair of inseminations, using semen from a control and an experimental bull, was conducted on the same farm and within the same month. (c) All possible pairs of inseminations on a given farm were recorded, with the reservation that data from an insemination involving a given cow were not used to make up more than one pair of inseminations.

By taking the above steps it was possible to minimize differences due to variations in farm management as practiced by individual farmers and to the varying ability of individual inseminators, since in most cases a given farm was served by a single inseminator. The utilization of pairs of inseminations made in the same month helped to reduce the effects of seasonal influences.

### RESULTS

*Chemical analyses.* The results of the analyses of the semen for ascorbic acid, total nitrogen and non-protein nitrogen are presented in table 3. Only the average values for the two groups are given, but the values for the different 12-wk. periods are included to serve as an indication of the variations from one period to the next. Apparently, the type of ration had no effect on the total and non-protein nitrogen levels. Variations in the level of these constituents in different ejaculates from the same bull indicate that the small differences shown in the table are not significant. Although the level of ascorbic acid in the semen shows a noticeable decrease over the experimental period, this is reflected in both groups and probably is not attributable to the rations fed.

The acid and alkaline phosphatase levels of the semen are given in table 4. Apparently, the levels of both of these semen constituents were slightly elevated in the case of the bulls on the all-roughage ration. Unfortunately, these determinations were started relatively late in the experiment, so that there were no data giving a comparison of the phosphatase levels of the semen of the two groups of bulls prior to the experiment. Furthermore, the phosphatase levels obtained on different ejaculates, even from the same bull, vary over a wide range, while one of the experimental Guernseys had such a low level of alkaline phosphatase that no appreciable activity could be obtained with three different ejaculates.

Examination of the data on volume of the first and second ejaculates indicated that there was no breed difference between the Guernsey and Holstein bulls in this respect. Comparisons of volumes before and during the experiment indicated that high volume bulls continued to produce large volumes despite the ration used. It was apparent that the experimental ration was without effect on ejaculate volume. The ejaculates averaged approximately 5.5 to 6.5 ml. per ejaculate with little or no difference between the first and second ejaculates.

Detailed data on the initial motility and density of ejaculates are not presented. However, the semen rating for each bull, as estimated on the basis of both initial motility and density of ejaculates taken during the last few months of the experiment, together with a similar rating for the pre-experimental period,

are given in table 2. From these data it appears that the all-roughage ration had no effect on these semen characteristics.

The average values for the duration of a motility rating greater than "1+" for semen from the two groups of bulls gave a difference between them of less than 12 hr. of storage time, which was not considered significant. The experimental ration apparently had no detrimental effect.

During the early part of the experiment, the young Guernsey bulls on the experimental ration showed considerable roughing of the hair coat, but this condition cleared up as the experiment progressed. One of the veterinarians employed by the breeding ring judged the bulls on the basis of "general thrift, vigor and condition" at the start and the close of the experimental period. A comparison of bulls on the basis of the above showed that there was no difference between the bulls fed the two rations.

It generally is believed that too much silage may result in "paunchiness" in bulls. The paunch- and heart-girths of each of the bulls were measured at the start and close of the experiment and these measurements are listed in table 2. In no case among the mature bulls was there any marked increase in paunch girth after 12 mo. on the all-roughage ration. Increases in the paunch girth of the younger bulls were the result of growth as evidenced by the simultaneous increase in heart girth, and by similar increases observed in the control animals.

The average fertility of the bulls in the two groups is summarized in table 2. The per cent non-returns for the Holsteins were 71.5 and 70.0 per cent for the control and the experimental animals, respectively, while the corresponding values for the Guernseys were 67.8 and 65.1 per cent. When considered from a practical aspect, this difference in the fertility of the two groups appears unimportant. This conclusion further was borne out by statistical analysis, the differences not being significant when tested by means of the "Chi-square" test (11).

#### DISCUSSION

The data concerning the phosphatase activity of the semen were insufficient to demonstrate any significant trend, but it is of interest to compare these results with the findings of Reid *et al.* (7) who reported that the levels of both acid and alkaline phosphatase in the semen of bulls receiving a complex concentrate mixture were "markedly elevated" above that of the semen from bulls receiving a simple concentrate mixture. A further discrepancy with our data lies in the relative amounts of acid and alkaline phosphatase. Reid and his co-workers reported that the mean level of alkaline phosphatase was considerably higher than that of acid phosphatase, while in the present study the opposite was found to be the case.

To date, no controlled experiments have been conducted to investigate to what extent silage can be used in the rations of bulls. Reid and his co-workers (8, 9) were able to show that the concentrate mixture used in bull rations can be made considerably less complex without any detrimental effect upon the composition of the blood or the semen quality.

Branton *et al.* concluded that 1 lb. of hay together with 0.4 to 0.5 lb. concentrate mixture daily per 100 lb. body weight was sufficient to meet the needs of bulls used for artificial insemination purposes.

Contrary to general beliefs, measurements of the paunch girth of the animals indicated that an all-roughage ration could be fed with silage at a level three times higher than that normally recommended without the development of "excessive middle."

Although not set up with this in mind, the above study serves to confirm the work of Branton *et al.* (2) who were able to show that, for the nutrition of bulls, "animal protein was not superior to the plant proteins" under the conditions of their experiment. When the results presented in this paper are studied in conjunction with those obtained by Reid *et al.* (9) and Branton *et al.* (2), it seems justifiable to conclude that, provided sufficient energy is supplied in the ration and the level of protein is adequate, the source of this protein is not of major importance.

Whereas the present study was conducted with bulls ranging from 14 mo. to 11 yr. of age, the question as to whether such an all-roughage ration can be fed to fast-growing bulls less than 1 yr. of age, was not answered by this experiment. Furthermore it is not known what the effects of this ration will be if it was fed over a period longer than 12 mo. The present study is being continued for another 12 mo. in order to obtain further information on this aspect of the problem.

In conclusion, it may be stated that the feeding of bulls, used for artificial breeding purposes, on a ration consisting solely of roughages seems to hold considerable promise. The adoption of such a feeding practice by artificial breeding rings should prove of considerable economic importance not only to the rings, but also to the dairy industry in general.

#### SUMMARY

A study has been made of the effects of an all-roughage ration including a high level of silage upon dairy bulls in a controlled experiment for a 12-mo. period.

Measurement of the ascorbic acid, total nitrogen and non-protein nitrogen content of semen indicated that there was no observable difference in the levels of these constituents in semen from bulls fed the all-roughage or the control ration. Although the levels of acid and alkaline phosphatases appeared slightly elevated in the semen of the bulls on the all-roughage ration, it was not possible to arrive at a definite conclusion as to the significance of the differences reported.

Regardless of the ration fed, the initial motility, density and volume of ejaculates, as well as the longevity of the spermatozoa in storage, were similar. The "over-all condition" and health of the bulls was maintained on the all-roughage ration. "Excessive middle" did not develop despite the feeding of high silage levels.

On the basis of fertility data it appears that the two rations were equally efficient in maintaining the reproductive ability of the bulls.

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## PARTITION OF ORALLY ADMINISTERED RADIOACTIVE PHOSPHORUS IN THE BLOOD AND MILK OF THE DAIRY COW<sup>1</sup>

P. SAARINEN<sup>2</sup>, C. L. COMAR<sup>3</sup>, S. P. MARSHALL AND GEORGE K. DAVIS

*Florida Agricultural Experiment Station, Gainesville*

The present knowledge of the blood precursors of phosphates in milk is based mainly on data obtained by a comparison of the phosphorus content of arterial and mammary venous blood. These data (3, 4, 9, 10, 11, 12, 13, 17, 18) seem to show that the mammary gland removes only plasma inorganic phosphate from blood. Consequently, this has been considered the sole precursor of all phosphorus occurring in the various compounds in milk. Since exchange of phosphates between blood plasma and tissues is very rapid and the equilibrium between inorganic phosphates and organic esters also is labile, the slightest excitation of test animals may affect the results obtainable with the arterio-venous difference technique. Therefore, additional information regarding phosphorus metabolism of the mammary gland was considered desirable.

Aten and Hevesy (1), working with goats, were the first to use labeled (radioactive) phosphorus in milk formation studies. The data presented by these workers appear to be in good agreement with the results obtained with A-V difference technique. However, at one time interval in these experiments, the specific activity of the main phosphorus fractions in milk reached a higher level than the simultaneous value of plasma inorganic phosphorus, the fraction which showed the highest specific activity in blood. A direct comparison of these values was difficult, because the effect of the subcutaneously administered radioactive phosphate lasted a relatively short time, and no definite conclusion could be drawn by comparing the simultaneous values of the specific activity of plasma inorganic phosphates and milk phosphates in different fractions. To explain the differences in the simultaneous specific activity values in blood and milk, it was assumed that 3 to 4 hr. are required before blood plasma phosphates are excreted in the milk.

Very few additional data (2, 7) regarding the partition of radioactive phosphorus in blood and milk have been published since the above work. It appeared desirable to repeat this work in such a way as to reduce the rate of change in specific activity values and obtain these values over a longer period of time. This was accomplished by oral administration of radioactive phosphorus instead of subcutaneous or intravenous injection.

### EXPERIMENTAL METHODS

A Jersey cow (no. 982UF) from the Florida Agricultural Experiment Station dairy herd, weighing 857 lb. and producing about 20 to 22 lb. of milk per day

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<sup>2</sup> Permanent address: University of Helsinki, Finland.

<sup>3</sup> Permanent address: UT-AEC Agricultural Research Program, Oak Ridge.

was chosen for the experiment. During the entire experiment, the cow was fed regularly twice a day according to the usual practices in the herd. The mixed concentrates (17 per cent total crude protein) contained the usual mineral additions of 1 per cent each of common salt, marble dust ( $\text{CaCO}_3$ ) and steamed bone-meal. She grazed on a fertilized pasture. On December 11, 1947, the cow was milked at the usual time, *i.e.*, 3:30 to 3:40 p.m. On December 12, at 4:45 a.m., before the cow had consumed any feed, labeled disodium phosphate containing 247.5  $\gamma$  of phosphorus with an activity of approximately 3.1 millicuries<sup>4</sup> was administered orally in about 200 ml. of water. The cow was milked the first time at 5:30 to 5:38 a.m. Because of the unavailability of pituitrin or oxytocin, the amount of milk secreted during the 59 min. after administration of the isotope was considered to be in proportion to the interval between milkings. After the first milking, the cow was milked three times at 4-hr. intervals. On the following days, the cow was milked twice a day at the usual milking times. The first blood sample was drawn on December 12, at 5:44 a.m. and subsequently, immediately after every milking. The samples were taken from the coccygeal artery using Saarinen's (16) technique.

Radioactivity was measured in solution with a dipping-type Geiger counter, using aliquots from the solution administered to the animal as standards according to usual procedures. Activity values are expressed in terms of micrograms of labeled phosphorus, whereas specific activity values are expressed as micrograms of labeled phosphorus per gram of total phosphorus. Counts were made from 15-ml. aliquots either directly or after proper dilution of the sample. Only the first milk sample was concentrated before the reading was taken.

The total amount of phosphorus in each fraction was determined colorimetrically using the method of Kuttner, *et al.* as modified by Saarinen (15) and the blood plasma acid soluble phosphates were extracted by the procedure therein described. Blood plasma phospholipids were extracted according to Bloor's (5) procedure and purified by redissolving in dry ether. Casein was precipitated from skim milk after the method of Brereton and Sharp (6). Milk serum phosphorus was determined in an aliquot of the casein filtrate.

#### RESULTS AND DISCUSSION

The values for total phosphorus and labeled phosphorus in blood and milk samples are presented in tables 1 and 2. It will be noted in table 1 that the blood plasma acid soluble phosphorus was distinctly radioactive in the first blood sample, taken 59 min. after the oral administration of the  $\text{P}_{32}$  phosphate. In this sample and in the second blood sample, taken about 4 hr. later, the blood plasma phospholipids did not show any activity, *i.e.*, the orally administered radioactive phosphorus was at first absorbed into the blood stream in an acid soluble form. When these values are compared with the results presented in table 2, it will be observed that in the milk secreted during the 4-hr. period preceding the drawing of the second blood sample, the milk serum acid soluble phosphorus was consider-

<sup>4</sup>The radioactive phosphorus was obtained from the Oak Ridge National Laboratory on authorization by the U. S. Atomic Energy Commission.



ably radioactive, and, likewise, the casein phosphorus. This indicates that the casein phosphorus had originated from the acid soluble phosphate fraction of the blood and not from the phospholipid fraction, but does not necessarily support the common view that the inactive phosphorus of phospholipids is not utilized simultaneously by the mammary gland.

The other results presented in table 1 show that the actual amounts of  $P_{32}$  in blood and plasma are at about the same level. The activity in milk is from 10 to 20 times higher than that of the blood and plasma. This is due mainly to the larger amount of total phosphorus in milk.

The values for blood and plasma labeled phosphorus in table 1 show two maxima. The first occurs in plasma 5 to 6 hr. after administration of the phosphate and the second in sample 6 at about 30 hr. later. The first maximum is due entirely to the activity of the acid soluble phosphates, but the second is due

TABLE 1  
*Labeled and total phosphorus in blood*

Sample no.	Date and sampling time	Whole blood labeled P	Blood plasma labeled P	Plasma acid soluble P		Plasma phospholipid P	
				Labeled P	Total P	Labeled P	Total P
		( $\gamma/100$ ml.)	( $\gamma/100$ ml.)	( $\gamma/100$ ml.)	( $mg./100$ ml.)	( $\gamma/100$ ml.)	( $mg./100$ ml.)
1	Dec. 12, 5:44 a.m.	0.00023	0.00027	0.00012	9.81	0.00000	7.48
2	Dec. 12, 9:40 a.m.	0.00550	0.00667	0.00703	10.10	0.00000	8.42
3	Dec. 12, 1:36 p.m.	0.00562	0.00581	0.00533	11.22	0.00048	8.04
4	Dec. 12, 5:50 p.m.	0.00381	0.00303	0.00273	11.50	0.00056	7.67
5	Dec. 13, 5:45 a.m.	0.00733	0.00733	0.00517	11.87	0.00301	7.67
6	Dec. 13, 3:45 p.m.	0.00815	0.00803	0.00517	11.59	0.00296	6.84
7b	Dec. 14, 3:45 p.m.	0.00775	0.00724	0.00397	12.16	0.00552	6.99
8b	Dec. 15, 3:45 p.m.	0.00601	0.00567	0.00188	12.16	0.00487	7.48

to activity of both acid soluble and phospholipid fractions, although the most marked increase in the phospholipid fraction occurred still later (samples 7b and 8b).

On the basis of the form of the blood labeled phosphorus, it may be assumed that the first increase was due to absorption of a portion of the  $P_{32}$  that passed directly to the omasum or abomasum or was absorbed from the rumen. The second increase occurred after the feed given with the tracer should have been largely absorbed (8, 14) and probably followed normal absorption, although interaction with bone and other tissues may have been involved. Although nearly simultaneous, the fluctuations were wider in the plasma than in the blood suggesting a diffusion equilibrium of soluble phosphorus compounds between plasma and erythrocytes.

As shown in table 2, the activity of the skimmilk was considerably higher than that of whole milk during the first 58 hr. of the experiment. While the diluting effect of the milk fat would explain part of this difference, the initial

TABLE 2  
*Labeled and total phosphorus in milk*

Sample no.	Date and milking time	Milk (g.)	Whole milk labeled P ( $\gamma/100$ mL.)	Skimmilk labeled P ( $\gamma/100$ mL.)	Acid-soluble serum P in skimmilk		Casein P in skimmilk	
					Labeled P	Total P	Labeled P	Total P
					( $\gamma/100$ mL.)	(mg./100 mL.)	( $\gamma/100$ mL.)	(mg./100 mL.)
1	Dec. 12, 5:30-5:38 a.m.	4,495	0.0000585			92.2		24.3
2	Dec. 12, 9:30-9:40 a.m.	1,317	0.0223	0.0341	0.0285	102.9	0.0036	23.9
3	Dec. 12, 1:30-1:36 p.m.	1,589	0.0704	0.1041	0.0819	104.7	0.0168	25.2
4	Dec. 12, 5:30-5:40 p.m.	1,861	0.0957	0.1219	0.0995	101.0	0.0242	25.2
5	Dec. 13, 5:30-5:40 a.m.	4,585	0.1122	0.1219	0.0875 <sup>a</sup>	110.3	0.0242	23.9
6	Dec. 13, 3:30-3:40 p.m.	4,086	0.1050	0.1242	0.0928	98.2	0.0210 <sup>a</sup>	21.5
7a	Dec. 14, 5:30-5:40 a.m.	6,265	0.0909	0.1018	0.0773	107.2	0.0196	25.1
7b	Dec. 14, 3:30-3:40 p.m.	2,479	0.0869	0.0933	0.0686	99.7	0.0175	23.6
8a	Dec. 15, 5:30-5:40 a.m.	6,538	0.0632 <sup>a</sup>	0.0522 <sup>a</sup>	0.0480 <sup>a</sup>	101.5	0.0137	22.9
8b	Dec. 15, 3:30-3:40 p.m.	3,541	0.0497	0.0458	0.0464	107.2	0.0121	23.7
9a	Dec. 16, 5:30-5:40 a.m.	5,085	0.0352	0.0406		95.9	0.0104	24.8
9b	Dec. 16, 3:30-3:40 p.m.	3,541	0.0380					
10a	Dec. 17, 5:30-5:40 a.m.	5,403	0.0294					
10b	Dec. 17, 3:30-3:40 p.m.	3,723	0.0249					
11a	Dec. 18, 5:30-5:40 a.m.	5,085	0.0204					

<sup>a</sup> Variable readings.

differences probably are due to the slow increase in the activity of milk phospholipid phosphorus as noted previously in this laboratory. During the period from 60 to 80 hr. after the beginning of the experiment, samples 8a and 8b, the whole milk showed higher activity than the skimmilk. While some of the duplicates were somewhat variable, the variations were not so great as to invalidate the conclusions.

It was during this period that the blood plasma phospholipids showed a very high activity (table 1, samples 7b and 8b), and it was suspected that, contrary to general belief, the blood plasma phospholipids had passed into the milk.

The activity of the milk phospholipid phosphorus fraction was determined on the samples following 8b, but the relative activity of whole milk and skimmilk were nearly the same and the blood plasma phospholipid phosphorus had decreased to a point where the results could not be used to check the above observation. To evaluate the transfer of blood plasma phospholipids in the mammary gland, it probably will be necessary to follow the path of intravenously administered  $P_{32}$  labeled bovine blood phospholipids.

When considered on the basis of micrograms of labeled phosphorus per gram of total phosphorus, the data in tables 1 and 2 demonstrate that the phosphorus in both the casein and milk serum fractions must have originated in the acid soluble phosphorus fraction of blood plasma rather than in the phospholipid fraction. Aten and Hevesy (1) noted that 1 to 3 hr were required for casein formation. This and the fact that during the first 34 hr. of the experiment the specific activity of casein phosphorus expressed as micrograms of labeled casein phosphorus per gram of total casein phosphorus increased smoothly to a maximum, while the specific activity of milk serum phosphorus showed marked variations, indicates that part of the milk serum phosphorus may originate from a different source than does casein phosphorus.

While the esterified phosphates of blood were not determined separately, the specific activity of phosphorus in the milk serum and casein reached and maintained a level so much higher than the blood plasma acid soluble phosphate fraction that the difference in milk serum and casein phosphate levels could hardly have been affected by the ester phosphates even if they were inactive.

These results are in general agreement with those of Aten and Hevesy (1) who reported that the specific activity of the milk inorganic and casein phosphorus was about 1.7 times higher than that of the blood plasma inorganic phosphorus at 4.25 hr. after subcutaneous administration. From the results in tables 1 and 2, it can be seen that after about 5 hr. the specific activity of the milk serum phosphorus and of the casein phosphorus was always higher than that of the plasma acid soluble phosphorus, although the general trend of the three values was similar. It was only after 59 hr. that the specific activity of the milk serum phosphorus fell to the highest value obtained for plasma acid soluble phosphorus.

Since it is unlikely that this could be due to a differential behavior of the isotopes  $P_{32}$  and  $P_{31}$ , a more probable explanation would be that plasma inorganic phosphorus is comprised of two or more forms with differing specific ac-

tivities and that there exists a preferential absorption by the mammary gland of the higher specific activity fraction.

The phosphorus of certain labile phosphate esters, such as the acye phosphates, is included in the inorganic phosphorus as usually determined. Consequently, it probably will be necessary to study the true inorganic phosphates and the labile organic phosphates before definite conclusions can be drawn concerning the blood precursors of the acid soluble and casein phosphorus of milk.

#### SUMMARY

When phosphorus isotope  $P_{32}$  was given orally to a cow in mid-lactation, the blood showed a marked activity after 59 min., mainly due to the activity of blood plasma acid-soluble phosphorus fraction. Later, two activity maximums were noted in both whole blood and blood plasma; the first appeared about 5 to 6 hr. after the beginning of the test and the second one about 30 hr. later. During the first of these periods, only the acid-soluble phosphorus fraction in plasma was labeled. The blood plasma phospholipid phosphorus fraction did not show any activity until several hours later. The increase in the specific activity of phospholipid phosphorus fraction also was much slower than in the plasma acid-soluble phosphorus fraction. The comparison of the specific activity of phosphorus in different blood and milk fractions at different periods following administration of  $P_{32}$  shows clearly that both the acid-soluble phosphorus in the milk serum and the casein phosphorus originate from the blood plasma acid-soluble phosphorus fraction and not from the phospholipid phosphorus fraction. On the basis of the proportionally high activity of both the casein phosphorus and the acid-soluble phosphorus in milk serum, it is considered that possibly only one fraction of the phosphates usually determined as blood plasma inorganic phosphates serves as the main precursor of the phosphorus in milk. There was some evidence to indicate that blood plasma phospholipids also may be removed from the blood by the mammary gland.

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## THE EFFECT OF STERILE COPULATION ON TIME OF OVULATION IN DAIRY HEIFERS<sup>1</sup>

GERMAIN B. MARION, VEARL R. SMITH, T. E. WILEY<sup>2</sup> AND G. R. BARRETT

The females of most species of mammals ovulate spontaneously, whereas some species ovulate only after copulation or some other form of sexual excitement. Cattle ovulate spontaneously but differ from most other species in that ovulation does not occur regularly until postestrus. There has been considerable speculation as to the effect of copulation on the time of ovulation in dairy cattle, especially since the extensive adoption of artificial breeding and the possible lack of sexual stimulation by this process of breeding. There are no published data concerning the effect of copulation on time of ovulation in the bovine. Marshall (8) was of the opinion that coitus was necessary for ovulation in sheep towards the end of the normal breeding season. Comprehensive studies by McKenzie, *et al.* (7) showed that sterile copulation had no effect on time of ovulation in ewes, but did shorten the estrual period.

This study was undertaken to determine the effect of sterile copulation on time of ovulation in the bovine and on other phenomena related to estrus.

The time interval between the end of estrus and the release of the ovum in dairy animals has been determined by a number of investigators. Brewster and Cole (3) found this interval to be 14.5 hr. for cows and 11.5 hr. for heifers. Nalbandov and Casida (9) recorded data on 72 estrual periods of grade cows and found that the time of ovulation from the end of heat normally varied from 10 to 18 hr., while Asdell (1) reported a range of 13.5 to 15.5 hr. After a comprehensive study, Trimberger (11) reported that the average ovulation time of cows was 10.7 hr. and of heifers 10.2 hr. after the end of estrus.

Hammond (6) observed the sexual cycles of three heifers and noted that the length of the cycle was decreased following estrus in which the heifers were serviced by a vasectomized bull. Two of the three heifers went out of heat more quickly following copulation than if service was not permitted. Chapman and Casida (5) stated that clinically normal cows which did not conceive to service of fertile bulls had longer subsequent estrual cycles than those which were not serviced.

### EXPERIMENTAL METHODS

Thirty heifers from the University of Wisconsin herd, consisting of 21 Holsteins, 5 Guernseys, 3 Jerseys and 1 Brown Swiss, were used for this study. The heifers varied in age from 12 to 18 mo. and were confined to pasture lots during the experimental period, which was from June 16 to October 4. The heifers were

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<sup>2</sup> Present address: Cornell University, Ithaca, N. Y.

paired as evenly as possible according to age and breed, and the heifers of each pair were assigned arbitrarily to different groups.

The first estrus of the animals in group A was decided arbitrarily to be a control, and that of the B group and experimental period, so that contemporary information might be obtained during the course of the experiment. At the subsequent estrus, the treatments were reversed for both groups. Consequently, of the four estrual periods observed for each heifer, there were two experimental and two control periods. An experimental period differed from a control period only in that a heifer was mated with a vasectomized bull. An effort was made to mate the heifers during that phase of the estrual period when sexual receptivity was most intense. Four of the heifers were dropped from the experiment because of estrual abnormalities and another was sold.

The animals were observed for the onset of estrus twice daily at 6 a.m. and 6 p.m. The only acceptable criterion of estrus was willingness of the heifer to stand while being mounted by a bull or by another heifer. The many other external manifestations of estrus, such as the flow of mucus from the vulva, highly vascular, swollen vulval lips, general restlessness, bellowing, attempting to ride other females and ruffled hair coat over the tail head, were not considered conclusive evidence that a heifer was in heat. However, these signs were helpful in detecting approaching estrual periods. As soon as a heifer was noted in heat, she was confined to the barn.

The time of ovulation was determined by the rectal palpation method, which was utilized by Schmid (10). Later work has shown a close agreement between the findings by rectal palpation and post-mortem data (Brewster *et al.*, 4). The heifers were examined per rectum shortly after being noticed in heat. The size, position and tone of any follicles in either ovary were determined and recorded. If the follicle was turgid, the next examination was made after the animal went out of heat, from which time palpations were made at 2-hr. intervals until ovulation occurred. In cases where the follicle was found to be soft, rectal palpations at 2-hr. intervals were begun immediately. Time of ovulation was established as the midpoint between the last examination when the follicle was intact and the subsequent examination 2 hr. later when the follicle had collapsed. In one case the follicle ruptured during palpation, and since the follicle was noted to be flabby and apparently ready to rupture, the time of that ovulation was established as the time of palpation. Rectal palpations of all heifers were performed by two or more workers, each recording his observations independently of the other.

The end of estrus was determined by checking the heifers every 2 hr. with other females or with a yearling Holstein bull, or both. The heifers were aproned to prevent copulation when a bull was used. The midpoint between the last check when the heifer stood quietly for mounting and the subsequent check when mounting was not permitted was taken as the time when the heifer went out of estrus. Usually the heifers were checked again 2 hr. later to confirm the previous observation.

The switchback technique was utilized, so that a simple group comparison could be employed in the analysis.

## RESULTS AND DISCUSSION

The data showing the effect of sterile copulation on the time of ovulation are presented in table 1. The average time intervals between the end of estrus and

TABLE 1  
*The effect of sterile copulation on time of ovulation*

GROUP A				
Time from end of heat to ovulation				
	Control	Exptl.	Control	Exptl.
No. of estrual period—	1	2	3	4
Heifer no.	(hr.)	(hr.)	(hr.)	(hr.)
22	10.50	7.25	5.00	11.25
25	8.50	5.50	9.25	3.75
26	2.25	2.75	14.50	4.25
28	12.00	10.00	14.25	4.00
31	11.75	10.75	10.75	7.00 <sup>b</sup>
34	13.25	12.50	9.00	5.00
35	14.25	11.00	12.50	16.00
39	8.00	5.00	14.00	8.00
42	9.75	2.00	10.00	12.25
43	10.00	1.75	6.25	8.00
45	4.75	5.25	6.25	7.50
47	6.75	4.00	8.25	13.00
48	9.25	6.50	10.00	2.50
Mean =	9.31	6.48	10.00	7.88

GROUP B				
Heifer no.	Exptl.	Control	Exptl.	Control
15	10.00	12.75	11.75 <sup>a</sup>	8.75 <sup>b</sup>
16	10.00	8.75	13.50	9.00
18	7.75	8.75	12.00	10.50
20	8.25	6.75	2.00	12.00
21	8.00	8.50	11.00	12.75
23	4.75	15.50	4.50	8.00
27	4.75	5.50	6.75	8.25
29	5.25	14.50	10.00	16.00 <sup>b</sup>
30	2.00	6.00	8.25	6.25 <sup>b</sup>
40	6.75 <sup>c</sup>	7.00	11.00	10.50
44	14.00	13.00	6.50	12.50
46	12.00	9.50	7.75	12.75
Mean =	7.79	9.71	8.75	10.60

<sup>a</sup> 4th consecutive estrual period.

<sup>b</sup> 5th consecutive estrual period.

<sup>c</sup> Mean of a double ovulation.

## Summary of statistical analyses

$$\begin{aligned} \text{Group A} &= \frac{\sum X}{+ 155.75} & \frac{\sum X^2}{1788.94} & \frac{T}{3.220^{**}} \\ \text{Group B} &= - 68.75 & \frac{2522.06}{T_{23} \text{ for } 23 \text{ d.f.} = 2.807. (P = < .01)} \end{aligned}$$

the rupture of the follicle were 7.73 hr. and 9.91 hr. for the experimental and control groups, respectively. The interval for the control period was similar in length to that obtained by Trimberger (10). The difference of 2.18 hr. was



highly significant. The method of analysis used was that of Brandt (2). The values  $O_1$ ,  $O_2$ ,  $O_3$  and  $O_4$  were assigned to estrus periods 1, 2, 3 and 4, respectively, for both groups, and the formula  $O_1 - 30_2 + 30_3 - O_4$  was utilized to determine the individual variates. The results of the tabulations are shown at the foot of table 1.

The individual ovulation intervals varied greatly for any particular heifer, and there was a wide range in the time of ovulation for both the control and the experimental periods. For the control periods the range was from 2.25 to 16.00 hr., while for experimental periods it was from 1.75 to 16.00 hr. However, when the average ovulation intervals for the control and experimental periods were compared, the variation was small. Although no special effort was made, met-estrum bleeding was observed to be associated with 60 of the 100 estrual periods. A bloody discharge was observed to occur from one heifer before ovulation during a control estrual period.

Table 2 presents the data showing the effect of sterile copulation on the length

TABLE 2  
*Effect of sterile copulation on length of estrual period*

GROUP A				
Treatment—	Control	Exptl.	Control	Exptl.
No. of estrual period —	1	2	3	4
Mean (hr.)	21.92	19.62	20.48	17.79
GROUP B				
Treatment —	Exptl.	Control	Exptl.	Control
No. of estrual period —	1	2	3	4
Mean (hr.)	18.79	19.94	16.67	22.10
Experimental			Control	
Over-all mean (hr.)	18.22		21.11	

of time the heifers remained in heat. Since only two examinations were made daily for the detection of heat, the error in the time of the onset of heat may be quite large. However, since both experimental and control groups were handled similarly, this error should be balanced. It was noted that heifers in heat often stood quietly to be mounted by a bull after they no longer would stand for another female. Therefore, a bull was always used to check the termination of heat so that it could be determined as accurately as possible. The average length of estrus of all experimental periods was 18.22 hr., while the average for the control groups was 21.11 hr. The range in length of the experimental periods was 5.00 to 33.25 hr., and for the control periods, 6.00 to 41.00 hr. When the figures which were recorded as the length of each individual estrual period were treated statistically, using the same technique as before, there was no significant difference between the length of the experimental estrual periods and the length of the control periods.

Coitus was found to have no effect on the length of the succeeding cycle. The average length of the 50 estrual cycles following the estrual period during which copulation was permitted was 21.8 days, while the 50 cycles following non-copulatory estrual periods averaged 21.7 days in length. This observation is not in agreement with that of Hammond (6) or of Chapman and Casida (5).

#### SUMMARY

The effect of sterile copulation on the time of ovulation was observed on 25 heifers representing four dairy breeds.

The heifers ovulated, on an average, at 7.7 hr. following the end of estrus when serviced by a vasectomized bull, as compared to 9.9 hr. when not serviced. The difference was highly significant.

The average length of non-serviced estrual periods was 21.1 hr., compared with 18.2 hr. for estrual periods during which copulation occurred. Statistical analysis showed the difference to be insignificant.

Sterile copulation had no effect on the length of the subsequent estrual cycle.

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# THE DETERMINATION OF PROTEIN SULFHYDRYL GROUPS WITH IODINE AND O-IODOSOBENZOATE BY AN AMPEROMETRIC TITRATION.<sup>1</sup>

BRUCE L. LARSON<sup>2</sup> AND ROBERT JENNESS

*Division of Agricultural Biochemistry, University of Minnesota, St. Paul 1*

Iodimetric titrations have been used extensively to determine reducing matter in many biological systems. Iodine itself in acid media reacts not only with such low molecular weight reductants as ascorbic acid and glutathione but also with some proteins. Hess and Sullivan (6) found that the amount of iodine reduced by native proteins in acid solution corresponded to their cysteine contents as determined by colorimetric analysis of acid hydrolysates. It is fairly well established that there are several degrees of reactivity or availability of the sulfhydryl groups of proteins. Anson (1) found that iodine will react with all of the sulfhydryl groups of native egg albumin and iodoacetamide with about half of them but that the reagents nitroprusside and acid ferrieyanide show a negative test.

o-Iodosobenzoate was first proposed by Hellerman *et al.* (8) as an oxidant for the quantitative determination of protein sulfhydryl groups. Hellerman *et al.* (7) determined by inhibition tests that o-iodosobenzoate oxidizes only part of the sulfhydryl groups of the enzyme urease, but they also showed that it oxidizes cysteine, glutathione and apparently the sulfhydryl groups of guanidine-denatured proteins quantitatively to the respective disulfide compounds.

In preliminary experiments with egg albumin and  $\beta$ -lactoglobulin, using the o-iodosobenzoate procedure (not in guanidine), essentially the same titration value was obtained for the native as for the guanidine-denatured protein. The fact that iodine apparently reacts with all of the sulfhydryl groups of native proteins and o-iodosobenzoate with only the more reactive or accessible ones, such as are present in guanidine denatured egg albumin (8), suggested that in titrating proteins by the o-iodosobenzoate method part of the oxidation may be due to iodine, since the excess o-iodosobenzoate is determined by liberation of iodine from iodide ion in an acid medium. Apparently the stoichiometry of the oxidation, whether produced by iodine or o-iodosobenzoate, is similar.

The presence of proteins may obscure the iodine end point, whether determined by the blue starch-iodine color or by the yellow color of iodine itself. Since this difficulty is encountered not only in direct iodine titrations but also in the o-iodosobenzoate method, a more precise method of determining the end point

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was sought. This paper reports the use of an amperometric adaptation of the "dead stop" titration of Foulk and Bawden (4) for this purpose. This method depends on the depolarizing action of iodine on a polarized platinum cathode and is especially applicable for the determination of small amounts of iodine in opaque sols such as milk.

#### METHOD

The apparatus employed was similar to that usually used for the "dead stop" titration (4, 13). A potential of 10 to 20 mv. (usually 10 mv.) was maintained across a pair of 5-cm. bright platinum electrodes immersed in the solution. A galvanometer having a sensitivity of 0.02 microamperes per mm. was included in the circuit to measure the current which flows while the cathode is being depolarized by the iodine. Iodide ion keeps the anode depolarized throughout the titration. Stirring was accomplished by means of a magnetic stirrer.

The detailed procedure for the titration is as follows: Two to 20 ml. of solution at a pH of 6.6 to 7.0 (protein sols containing 0.25 to 3.0 g. per 100 ml. may be used) are introduced into a 100-ml. beaker, followed by 4.0 ml. of approximately 0.005 *N* sodium *o*-iodosobenzoate from a 5-ml. burette graduated to 0.01 ml. The mixture is gently stirred for 2 to 3 min. and during this time a flask containing 5 ml. of 1*N* HCl (or enough to give a final pH of 1.5 to 2.0), 5 ml. of freshly diluted 3 per cent KI and 10 ml. of standardized freshly diluted 0.002 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> is prepared. The contents of this flask then are poured and rinsed into the beaker and the volume made to approximately 100 ml. with distilled water.<sup>3</sup> With constant stirring the mixture is titrated with more of the 0.005 *N* *o*-iodosobenzoate until free iodine is present as indicated by a slight permanent deflection of the galvanometer. More of the solution is added in increments and the volumes added (including the original 4.0 ml.) are plotted against the galvanometer readings. Extrapolation of the plot to zero current flow gives the end point. A blank is run on the solvent (*i.e.*, water, buffer, etc.) in exactly the same manner and this constitutes a standardization of the *o*-iodosobenzoate against the standard thiosulfate. When iodine is used directly as the oxidant, the sol is first acidified, KI added and iodine or iodate titrated into the solution. Calculations of cysteine percentages were made on the assumption that sulphydryl groups are oxidized to the disulfide (6, 8).

#### RESULTS AND DISCUSSION

In figure 1 is shown the titration of casein sols of two concentrations in phosphate buffer. Extrapolation of the curves to zero current flow shows that no *o*-iodosobenzoate or iodine was reduced. Identical results are obtained using a direct iodine titration. The apparent reducing capacity of casein if the starch-iodine end point had been used is illustrated clearly in figure 1. Iodine does not form a visible complex with starch until the iodine normality is  $1-10 \times 10^{-6}$  *N*,

<sup>3</sup> This large volume was found necessary with casein-containing sols as milk. If the casein sol is not first diluted up with 30 or 40 ml. of water before the acid and iodide are added, a precipitate forms which clogs up to the electrodes and interferes with the readings.

depending somewhat on the concentration of iodide ion and the type of starch used (10, 11). In the present investigation, titration of a purified amylose starch fraction produced a blue color at a concentration of  $2.5 \times 10^{-6}$  *N* free iodine (galvanometer reading = 50 mm.). However, casein apparently adsorbs part of the iodine from the aqueous phase and, since the blue color with starch will not appear until the concentration of free iodine has reached  $2.5 \times 10^{-6}$  *N*, the casein would appear to have a considerable reducing capacity (in this case amounting to 0.15 per cent cysteine) if starch were used to detect the end point. All proteins tested except gelatin exhibited the ability to decrease the slope of the plot by this adsorptive process. The iodine adsorbed apparently is held reversibly, since it can be removed readily by a back titration with thiosulfate.

The slope of the plot depends on the final volume of the solution which

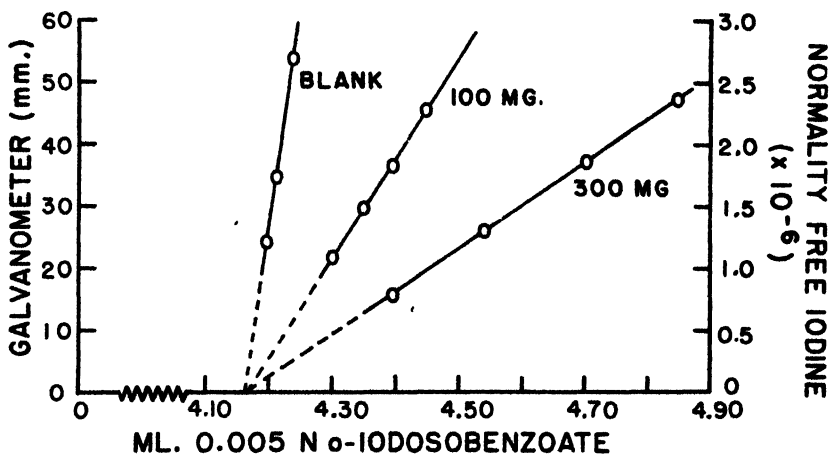


FIG. 1. Titration of casein with o-iodosobenzoate, showing the effect of protein concentration on the amount of iodine required to yield a given galvanometer reading. The normality of free iodine corresponding to galvanometer readings was computed from the increments of o-iodosobenzoate added in the blank titration.

determines the normality of iodine and also on the rate of stirring which affects the diffusion of iodine to the electrodes. The magnetic stirrer employed produced a constant rate of stirring for a given titration but some variability occurred between titrations. It has been observed repeatedly that duplicate titrations extrapolate to the identical end point even though the slopes differ. Consequently, while it is necessary to maintain a constant stirring rate during a given titration, it is not essential to do so from one titration to another.

In order to determine the specificity of the method for protein groups, several amino acids and proteins were titrated by the o-iodosobenzoate method and by the direct iodine titration. The plots for casein and gelatin, which do not contain sulfhydryl groups, extrapolate back to the same point as the blank; those for egg albumin and  $\beta$ -lactoglobulin extrapolate to values characteristic of the protein and proportional to the quantity present. An example of the titration as applied

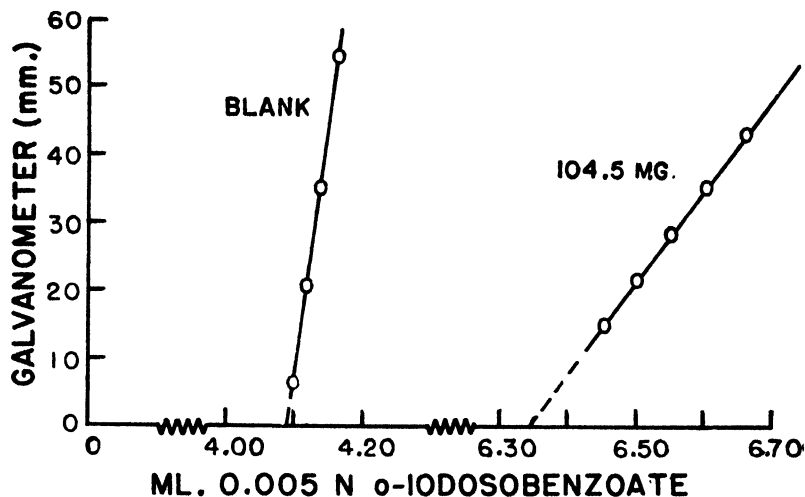


FIG. 2. Titration of 104.5 mg. of crystalline  $\beta$ -lactoglobulin. The calculated normality of the *o*-iodosobenzoate is 0.004988 since 10 ml. of 0.002040 *N* sodium thiosulfate was used in the blank or standardization titration. The difference in titer between the blank and the  $\beta$ -lactoglobulin sol shows that the  $\beta$ -lactoglobulin has reduced 0.0112 m.eq. of oxidant which is equivalent to 1.30% cysteine.

to  $\beta$ -lactoglobulin is given in figure 2. The reducing capacities of the proteins and amino acids, calculated as cysteine percentage, are presented in table 1. In agreement with Hellerman *et al.* (7, 8) these results indicate a stoichiometric

TABLE 1  
*Reducing capacity of various proteins and amino acids*

Protein or amino acid <sup>a</sup>	Reducing power	
	Direct iodine titration	<i>o</i> -Iodosobenzoate titration
		(as % cysteine)
Casein <sup>b</sup>	0.00	0.00
Gelatin <sup>c</sup>	0.00	0.00
Egg albumin <sup>d</sup>	1.51	1.12
$\beta$ -Lactoglobulin <sup>e</sup>	1.30	1.30
		(m.eq./m.eq. cysteine)
Cysteine <sup>f</sup>	3.42	1.00
Glutathione <sup>g</sup>	1.03	0.94
Amino acid mixture <sup>h</sup>	0.00	0.00
Cysteine <sup>f</sup> + amino acids <sup>h</sup>	3.45	0.99

<sup>a</sup> All in phosphate buffer, pH 6.6,  $\mu=0.1$ .

<sup>b</sup> Prepared according to the method of Van Slyke and Baker (14).

<sup>c</sup> Difco Bacto brand.

<sup>d</sup> Recrystallized four times according to the method of Kekwick and Cannan (9).

<sup>e</sup> Crystallized according to the method of Bull (3).

<sup>f</sup> Pfanstiehl reagent grade (fresh supply).

<sup>g</sup> Elmer and Amend C.P. grade (old supply).

<sup>h</sup> 5 mg. each of cystine, methionine, histidine, phenylalanine, tryptophan and tyrosine.

oxidation of cysteine and glutathione to the disulfides. This is a direct oxidation by the o-iodosobenzoate since the nitroprusside test of these two materials is abolished by o-iodosobenzoate alone. Direct iodine titration of cysteine carries the oxidation to further stages (12), but this effect does not occur with glutathione (15). Direct iodine titration of egg albumin evidently causes some over-oxidation which does not occur if o-iodosobenzoate first is allowed to react with the protein, but both methods give identical results for  $\beta$ -lactoglobulin. The sulfhydryl groups appear to be the only protein groups oxidized by these reagents.

The cysteine contents of egg albumin and  $\beta$ -lactoglobulin calculated from the results of the titration (o-iodosobenzoate method) on the basis of the assumption that the stoichiometry of the oxidation corresponds to the formation of the disulfide are in reasonable accord with values in the literature obtained by other

TABLE 2  
*The effect of pretreatment with o-iodosobenzoate on the reducing power of egg albumin and  $\beta$ -lactoglobulin*

Protein	Treatment	Reducing power	
		o-Iodosobenzoate and iodine reduced	o-Iodosobenzoate reduced (actual)
		(as % cysteine)	
$\beta$ -Lactoglobulin	Water + dialysis	1.28	
$\beta$ -Lactoglobulin	o-Iodosobenzoate + dialysis <sup>a</sup>	1.26	0.02
Egg albumin	Water + dialysis	1.10	
Egg albumin	o-Iodosobenzoate + dialysis <sup>a</sup>	0.98	0.12

<sup>a</sup> 0.40 m.eq. of o-iodosobenzoate added per gram of protein and the excess removed by exhaustive dialysis in Visking sausage casings against phosphate buffer pH 6.6,  $\mu=0.1$ .

methods (2, 5). This fact furnishes some justification for using this method of calculation. The results seem to represent the total sulfhydryl content of these proteins.

To determine accurately what proportion of the oxidation is caused by o-iodosobenzoate itself, egg albumin and  $\beta$ -lactoglobulin were treated with o-iodosobenzoate, exhaustively dialyzed against buffer and finally titrated. The results, given in table 2, show that o-iodosobenzoate reacts with few if any of the reducing groups of native  $\beta$ -lactoglobulin but with about 10 per cent of those of native egg albumin. Thus, the o-iodosobenzoate titration as applied to native proteins actually involves principally oxidation by the iodine formed upon acidifying the sol and adding iodide. The advantage of using the o-iodosobenzoate treatment on protein systems is that any very reactive sulfhydryl groups such as seem to be present in egg albumin will not be over-oxidized by iodine. Fresh milk proteins apparently do not contain such reactive groups, but there is evidence that they are formed by heat treatment of the milk serum proteins.

## SUMMARY

An amperometric adaptation of the "dead stop" titration technique has been applied to determine the sulfhydryl groups of proteins with o-iodosobenzoate and iodine. As applied to native proteins, the oxidation is largely produced by the iodine liberated in the course of determining the excess o-iodosobenzoate.

The method appears to be specific and quantitative for sulfhydryl groups, since any very reactive groups which might be overoxidized by iodine react first with the o-iodosobenzoate.

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# THE REDUCING CAPACITY OF MILK AS MEASURED BY AN IODIMETRIC TITRATION<sup>1</sup>

BRUCE L. LARSON<sup>2</sup> AND ROBERT JENNESS

*Division of Agricultural Biochemistry, University of Minnesota, St. Paul 1*

In recent years the reducing components of milk have been studied extensively in relation to various processing procedures. The capacity of acidified milk or of a deproteinized acid filtrate of milk to produce 2,6-dichlorophenolindophenol has been widely used as a measure of the ascorbic acid content. In addition to ascorbic acid, the sulfhydryl groups of the milk proteins must be considered in attempting to elucidate the reducing system. Neither nitroprusside (15) nor thiamine disulfide (5, 6) is reduced by fresh milk, but capacity to reduce these reagents is produced by heat treatment. Ferricyanide at pH 6.6 is reduced by milk at 50° C., the capacity being largely accounted for by the ascorbic acid and the proteins present (1). The capacity to reduce ferricyanide is augmented by heat treatment, mainly as a result of the production of reductants by sugar-protein interactions (1, 6).

Larsen *et al.* (12), using a modification of the *o*-iodosobenzoate method of Hellerman *et al.* (7, 8), found that the reducing power of sols of the serum proteins decreased upon heat treatment, particularly in the presence of air. These decreases tended to parallel the improvement produced by such heat treatment in the baking quality of serum protein preparations and of skimmilk itself. More recently, Larson and Jenness (13) modified the *o*-iodosobenzoate method by use of an amperometric detection of the end point. They demonstrated that when applied to native egg albumin and  $\beta$ -lactoglobulin the method involves chiefly oxidation of sulfhydryl groups by iodine liberated at pH 1.5 to 2.0 in the course of determining the excess *o*-iodosobenzoate, rather than by the *o*-iodosobenzoate itself at a pH of 6.6 to 7.0. This paper reports the application of the method of Larson and Jenness (13) to milk. The constituents of milk which exhibit reducing power in this method and some of the effects of heat treatment have been studied.

Gould (4), using a method adapted from that of Woodward and Fry (21) for determining the glutathione content of blood serum, reported that sulfosalicylic acid filtrates of milk had much higher reducing capacities in an iodate-iodine titration than could be accounted for by the ascorbic acid present but reached no definite conclusions as to the identity of other reductants. This finding may be interpreted in view of the results of the present investigation.

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## METHODS

The *o*-iodosobenzoate titrations were made on 10-ml. samples by the method of Larson and Jenness (13). Also, some titrations were made on sulfosalicylic acid filtrates with iodate according to the method of Gould (4). Ascorbic acid was determined by titrations with 2,6-dichlorophenolindophenol of metaphosphoric-trichloroacetic acid filtrates prepared according to Doan and Josephson (3). The dye was standardized against ferrous ammonium sulfate as recommended by Stewart and Sharp (19). Analyses for nitrogen distribution were performed by the method of Rowland (17), using a micro-Kjeldahl method that combines digestion with selenium oxychloride as suggested by Pepkowitz and Shive (16) (except that the perchloric acid was omitted) with the distillation and titration technique of Ma and Zuazaga (14).

## EXPERIMENTAL

*Constituents responsible for the reducing power.* The following experiments were made to determine the respective contributions of the fat phase, the coloidal phase and the materials in true solution to the reducing power as determined by the *o*-iodosobenzoate iodimetric procedure.

(a) *The fat phase.* The contribution of the fat phase was determined by comparison of the reducing capacities of whole milk, skimmilk and cream from a single original lot of fresh mixed milk collected from the separator at the University creamery. The data given in table 1 show definitely that the fat phase

TABLE 1  
*Reducing capacities of whole milk, skimmilk, cream and an emulsion of milk fat*

Material	Fat (%)	Reducing capacity		
		Iodimetric	Dye <sup>a</sup>	"Non-ascorbic" <sup>b</sup>
			(m. eq./l.)	
Whole milk	3.95	0.495	0.187	0.308
Skimmilk	0.01	0.477	0.178 <sup>c</sup>	0.299
Cream	50.0	0.533	0.083 <sup>c</sup>	0.450
Milk fat in gelatin	16.7	0.00		

<sup>a</sup> 2,6-dichlorophenolindophenol.

<sup>b</sup> By difference.

<sup>c</sup> Ascorbic acid contents of the skimmilk and cream are lower than would be expected, probably due to oxidation during and following separation.

as well as the plasma contributes to the non-ascorbic reducing capacity. On a volumetric basis the non-ascorbic reducing capacity of the fat phase is somewhat greater than that of the plasma, since the cream had the highest titration value. Since no reducing capacity was exhibited by an emulsion of milk fat in gelatin (16.7 per cent fat), it is logical to conclude that the reducing power of the fat phase involves the materials adsorbed on the fat globules. The contribution of the fat phase to the reducing power of whole milk is very small, however, because of its low concentration therein.

(b) *The proteins and dissolved constituents.* Fresh whole milk representa-

tive of an entire milking of a single cow<sup>3</sup> of the University herd was obtained at milking time. Fractionations were made by dialysis to determine the relative contributions of the dialyzable and non-dialyzable constituents. Furthermore, dialysis experiments were set up in which portions of milk serum were dialyzed against milk and buffer, respectively. Dialysis was performed by placing the materials in Visking sausage casings which then were equilibrated in the desired medium on an inclined rotating turntable in a room at 5° C. Volumes were measured carefully before and after dialysis, and all of the titrations were made about 26 hr. after milking. Details of the fractionations and treatments as well as the results are given in table 2. The titration results show that the reducing

TABLE 2  
*Reducing capacity of milk fractions*

Fraction no.	Material dialyzed	Treatment		Milk constituents in fraction	Reducing capacity <sup>a</sup>	
		Dialysis medium	Type of dialysis		Iodimetric (m. eq./l.)	Dye (m. eq./l.)
1	Whole milk	None	None	All	0.577	0.244
2	50 ml. whole milk	6 l. buffer <sup>b</sup>	Exhaustive <sup>c</sup>	Non-dialyzable	0.362	0
3	60 ml. water	1950 ml. milk	Equilibrium <sup>d</sup>	Dialyzable	0.227	0.247 <sup>e</sup>
4	30 ml. serum <sup>f</sup>	1950 ml. milk	Equilibrium	Serum protein and dialyzable	0.577	0.244
5	60 ml. serum	6 l. buffer	Exhaustive	Serum protein	0.360	

<sup>a</sup> Calculated to basis of original milk.

<sup>b</sup> Phosphate buffer pH 6.6,  $\mu = 0.1$ .

<sup>c</sup> Three 2-l. portions of buffer over 24 hr.

<sup>d</sup> Equilibrated for 24 hr.

<sup>e</sup> Titrations with dye made in presence of metaphosphoric-trichloroacetic acid coagulant.

<sup>f</sup> Serum prepared by precipitating casein from 100 ml. milk with 10 ml. 10% acetic acid and 10 ml. 1 M sodium acetate.

power of milk as determined by this method is the summation of the effects of certain dialyzable constituents and the serum proteins. The combined contributions of the fat and caseinate, represented by the difference between fractions 2 and 5 or between 1 and 4, are negligible. While the previous experiment demonstrated that the fat phase does have reducing power, it is present in too small an amount in whole milk to contribute significantly. Purified casein was shown in a previous paper (13) to have no reducing power. This has been further verified by titration of a caseinate sol prepared by centrifuging skim milk in the Sharples supercentrifuge and dispersing the caseinate gel in a milk dialysate prepared by dialyzing 300 ml. of distilled water against 10 gal. of raw skim milk. Such a preparation had a reducing power identical to that of the dialysate itself. The values for the reducing capacity of the milk serum protein in this sample

<sup>3</sup> The general picture obtained with this milk was confirmed with another lot of milk from a second cow.

are equivalent to about 0.053 m.eq. per gram or 0.64 per cent cysteine whether determined directly (fraction 5) or by difference (fraction 4 minus fraction 3). This figure is in close agreement with data published previously (12) on other preparations of the serum protein mixture.

Ascorbic acid undoubtedly predominates among the dialyzable reducing materials. Titration of milk dialysate with *o*-iodosobenzoate gives approximately the same value as is obtained by 2,6-dichlorophenolindophenol titration of the dialysate or of deproteinized milk.

*Variations in reducing capacity among samples.* Reducing titration values for a number of fresh and commercial samples of whole and skim milk are presented in table 3. These results exhibit considerable variability among samples,

TABLE 3  
*Reducing capacity of various samples of milk*

Sample <sup>a</sup>	Iodimetric	2,6-dichlorophenol indophenol	Non-ascorbic	As cysteine <sup>b</sup>
	(m. eq./l.)	(m. eq./l.)	(m. eq./l.)	(%)
<i>Whole milk:</i>				
1—1 hr. <sup>c</sup>	0.572	0.226	0.346	0.76
1—26 hr.	0.512	0.143	0.369	..
2—1 hr.	0.642	0.265	0.377	..
2—26 hr.	0.574	0.202	0.372	..
3—26 hr.	0.577	0.244	0.333	0.60
<i>Skim milk:</i>				
4—Fresh	0.640	0.280	0.360	.
5—Commercial	0.357	0.085	0.272	0.52
6—Commercial	0.388	0.073	0.315	0.54
7—Commercial	0.288	0.053	0.235	0.48
8—Commercial	0.341	0.067	0.274	0.66
9—Commercial	0.325	0.087	0.238	0.50
10—Commercial	0.335	0.085	0.250	0.55
11—Commercial	0.324	0.077	0.247	0.55

<sup>a</sup> Samples 1, 2, 3, and 4 were from single milkings of individual cows.

<sup>b</sup> Calculated as per cent cysteine in the serum protein.

<sup>c</sup> Time of titration after milking.

not only in the total iodimetric reducing capacity but also in the "non-ascorbic" category which represents the serum proteins. In general, the commercial raw skim milks, which contained very little ascorbic acid, tended to be lower in non-ascorbic reducing capacity, not only on the basis of milliequivalents per liter, but also when calculated as cysteine percentages of the serum proteins. The data for whole milk samples 1 and 2 were confirmed in an independent analysis by H. A. Harland of the Division of Dairy Husbandry, who also has observed a range of 0.219 to 0.334 m.eq. per liter in non-ascorbic reducing capacities on 20 samples of commercial raw whole milks comparable to that obtained by us for commercial raw skim milks. Gould's (4) data also are of the same order of magnitude, his non-ascorbic values ranging from 0.214 to 0.387 m.eq. per liter for 14 samples. Since Gould's method of titration is similar to ours, and the results correspond, it appeared likely that his treatment with sulfosalicylic acid did not entirely precipitate the serum proteins. The validity of this explanation

TABLE 4  
Reducing capacity and efficiency of sulfosalicylic acid as a protein precipitant

Sample	Reducing capacity			Nitrogen		
	Iodimetric o-Iodoso- benzoate	Gould Method	2,6-dichloro- phenol- indophenol	Non- cascin <sup>a</sup>	In sulfo- salicylic filtrates <sup>b</sup>	Non- protein <sup>a</sup>
	(m. eq./l.)			(mgm./100 ml.)		
Commercial skims						
12—No heat	0.324	0.297	0.077	112.	70.0	27.
—155° F.	0.246	0.216	0.057		57.8	
13—No heat	0.335	0.312	0.085	112.	70.0	26.
—165° F.	0.236	0.111	0.060		44.0	
14—No heat	0.341	0.260	0.067	106.	71.0	27.
—185° F.	0.206	0.025	0.047		33.4	
15—No heat	0.325	0.297	0.087	121.	71.0	30.
—195° F.	0.227	0.069	0.073		32.7	
Reconstituted Dry skim <sup>d</sup>						
16—Freeze-dried	0.290	0.295	0.025	126.6	76.6	31.5
17—Spray dried						
145° F.	0.258	0.203	0.023	128.5	74.6	33.3
190° F.	0.100	0.044	0.066	63.0	42.6	34.3

<sup>a</sup> According to method of Rowland (17).

<sup>b</sup> Filtrate prepared according to method of Gould (4).

<sup>c</sup> All heat treatments were of 30 min. duration.

<sup>d</sup> Reconstituted in the amount of 10 g./100 ml.

is attested by the data in table 4. Sulfosalicylic acid as used by Gould does not precipitate completely the proteins of unheated milk, but it does more nearly do so in the case of heated milks.

*Effect of heat treatments.* The change in reducing capacity produced by

TABLE 5  
Effect of heat treatment on the o-iodosobenzoate reducing capacity of milk, milk serum proteins, and crystalline  $\beta$ -lactoglobulin

Temperature for 30 min.	Reducing capacity as cysteine		
	Reconstituted skim milk <sup>a</sup>	Serum proteins <sup>b</sup>	$\beta$ -lactoglobulin <sup>b</sup>
(° C.)	(%)	(%)	(%)
	Heated in nitrogen, titrated immediately on cooling		
Control	0.58	0.69	1.30
78		0.65	1.03–1.10 <sup>c</sup>
	Heated in air, titrated shortly after cooling		
64		0.59	1.15
69	0.37	0.50	
73		0.32	0.72
78	0.20	0.28	0.63
83		0.17	
97	0.22	0.20	0.43

<sup>a</sup> Freeze-dried unheated milk reconstituted in the amount of 10 g./100 ml. The ascorbic acid content of this milk was negligible. Cysteine percentages calculated on basis of serum protein in unheated control.

<sup>b</sup> In phosphate buffer at pH 6.9,  $\mu = 0.1$ .

<sup>c</sup> Greater precautions to exclude air were taken in the case of the milk serum protein sol than for the  $\beta$ -lactoglobulin sol.

subjecting skimmilk (reconstituted freeze-dried nonfat dry milk solids), milk serum protein sol, and  $\beta$ -lactoglobulin sol to 30-min. heat treatments at temperatures of 64 to 97° C. is shown in table 5. These data confirm the previous report (12) that heat treatment decreases the sulfhydryl reducing capacity of milk serum proteins. Probably this change is due to oxidation, since it is largely prevented by excluding air from the sample during heating.

#### DISCUSSION

This study shows that the *o*-iodosobenzoate titration method as modified by Larson and Jenness (13) is applicable to milk and other dairy products. Indeed, it was partly with the object of applying the method to opaque solutions such as milk that the method was devised. The fact that the fat phase (but not the fat itself) reduces *o*-iodosobenzoate and/or iodine in this determination is interesting in view of the reports of Josephson and Doan (10) and of Townley and Gould (20) that the materials adsorbed on the fat globule are a source of heat-labile sulfides in milk.

While it is probable that the principal dialyzable reductant is ascorbic acid, no claim can be made that it is the only one. The *o*-iodosobenzoate titration of milk dialysate is approximately the same as the titration with 2,6-dichlorophenol-indophenol but it must be recognized that neither method is specific for ascorbic acid.

Considerable variability in the sulfhydryl content of the serum protein fraction of various samples of milk was observed.  $\beta$ -Lactoglobulin undoubtedly is the principal contributor to the reducing power of this fraction, since crystalline preparations reduce 0.104 to 0.110 m.eq. of iodine per gram (13). Thus, if  $\beta$ -lactoglobulin represented 50 per cent of the milk serum proteins, it alone would account for titration values in the range obtained for the latter. Electrophoretic analyses indicate that components having the mobility of  $\beta$ -lactoglobulin comprise at least 50 per cent of the proteins of milk serum (2, 18).  $\beta$ -Lactoglobulin constitutes the major portion of the classical "lactalbumin" fraction. It is not known definitely whether the variations in sulfhydryl content of the various serum protein samples reflect the relative amount of  $\beta$ -lactoglobulin present, although this is strongly suspected of being the case.

The results show definitely that Gould's iodate titration of a sulfosalicylic acid filtrate actually involved some protein sulfhydryl groups because the deproteinization was incomplete. These proteins are responsible for the reducing power which he observed over and above that of the ascorbic acid present. Undoubtedly, the "destruction" of a reducing system by heat to which he referred involved both decrease of the protein sulfhydryl groups and increased precipitability of the serum proteins by sulfosalicylic acid. Evidently, that fraction of the serum protein which is precipitated by sulfosalicylic acid from unheated milk contributes little to the reducing power since titrations by Gould's method yield results comparable to those obtained by the *o*-iodosobenzoate procedure.

The fact that the decreases in reducing capacity produced by heating are similar for milk serum protein sols,  $\beta$ -lactoglobulin and skimmilk again indicates that  $\beta$ -lactoglobulin probably is the principal constituent involved. Apparently,

heating activates the sulfhydryl groups so that they become susceptible to oxidation by molecular oxygen. This oxidation, however, does not necessarily mean formation of disulfides from the sulfhydryl groups, since Larsen *et al.* (12) could find no increase in the cystine content of heated milk serum proteins as determined on the hydrolysate by the method of Kassel and Brand (11), even though the apparent titer had decreased. An alternate explanation of the loss of titratable sulfhydryl groups on heat treatment is that the protein micelle unfolds upon heating and assumes upon cooling a new configuration such that the sulfhydryl groups are shielded from the action of the *o*-iodosobenzoate or iodine. In the light of recent evidence (13) indicating that iodine and not *o*-iodosobenzoate is the principal oxidant and that iodine oxidizes all the sulfhydryl groups of native or denatured proteins (9, 13), steric hindrance does not seem adequately to explain the loss of sulfhydryl groups, although it may be a minor factor. Even though the loss of sulfhydryl groups appears to be due to oxidation by molecular oxygen, neither the kinetics of these processes nor the conditions affecting them have been studied thoroughly, and thus the data of table 5 should be regarded as preliminary results which indicate the similarity of the changes occurring in  $\beta$ -lactoglobulin, the serum protein mixture and milk itself. These results may not represent the maximum oxidation, since indications have been obtained that further decreases occur upon holding the cooled sample for periods up to 48 hr. Presumably such factors as diffusion of oxygen into the sample and the temperature of holding influence the rate of oxidation. At present, any attempt to use non-ascorbic reducing capacity as an index of the extent of heat treatment to which a sample of market milk may have been subjected is premature. The observed variability in the non-ascorbic reducing capacity of samples of fresh milk and the fact that oxidation may continue at slow and variable rates after heating complicate the relationship.

#### SUMMARY

The reducing systems of milk have been studied by an iodimetric titration employing *o*-iodosobenzoate. The fat phase, the serum proteins and the dialyzable portion all exhibit reducing capacity in this method. Since milk fat emulsified in gelatin has no reducing capacity, the materials constituting the natural "fat globule membrane" must be responsible for reduction by the fat phase. Titrations of purified crystalline  $\beta$ -lactoglobulin indicate that it probably is the principal reducing constituent of the serum proteins. Undoubtedly, ascorbic acid is the chief dialyzable reductant.

Considerable variability was found in the reducing capacity of the serum proteins in various samples of milk; commercial raw milks tended to give lower values than fresh milks.

Sulfosalicylic acid as used by Gould does not precipitate quantitatively the serum proteins from raw milk, but the efficiency of precipitation is greater in heated milk. Thus, the decrease produced by heat treatment of milk in the iodimetric titration values of sulfosalicylic acid filtrates is due to both decreased reactivity of protein sulfhydryl groups and increased precipitability of the serum proteins. The similarity in decreases of the reducing capacity for skim-

milk, purified serum proteins and crystalline  $\beta$ -lactoglobulin again suggests that  $\beta$ -lactoglobulin is the principal reducing component of the milk proteins. The decrease in reducing titer upon heat treatment probably is due to oxidation by molecular oxygen, since heat treatment of deaerated samples in the presence of nitrogen produces little or no decrease.

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## STUDIES OF HEATED MILK

### III. MODE OF FORMATION OF CERTAIN FURAN COMPOUNDS<sup>1</sup>

STUART PATTON

*Department of Dairy Husbandry*

*The Pennsylvania Agricultural Experiment Station, State College, Pennsylvania*

The need exists for more fundamental information relating to chemical changes induced in milk by high temperature treatment. To augment present knowledge, research has been progressing with the objective of identifying the compounds produced in milk by high temperature treatment and to elaborate the mechanism of their formation.

The presence of furfuryl alcohol in skim milk heated to high temperature has been reported previously. It was suggested that lactose or ascorbic acid might serve as the origin of the compound (6). More recent work has precluded the possible action of ascorbic acid in this connection and reduced the essential components of the reaction to lactose and casein. Some additional observations were that no furfuryl alcohol is produced by heating aqueous systems of lactose and glycine, glucose and casein or galactose and casein, the principal end product being 5-hydroxymethyl-2-furfural in these instances. Small quantities of the latter compound were shown to be present also in heated skim milk (5).

Excepting the isolation of furfuryl alcohol from coffee brew (8), there appears to be no information in the literature concerning the presence or mode of formation of this compound by heating food stuffs. Thus, the potential significance of furfuryl alcohol in the heat degradation of milk, its possible relationship to hydroxymethylfurfural and the decomposition of sugars warranted further study.

#### EXPERIMENTAL

The experimental procedure involved a uniform heat treatment of milk samples and simplified systems by autoclaving for 2.5 hr. at 127° C., unless otherwise indicated. The pH values of these samples before and after autoclaving were determined with a Beckman model M instrument employing a glass electrode. Preparation and ethyl ether extraction of the autoclaved samples have been described (5). The components of the ether extract, following removal of the solvent on a warm water bath, were separated by vacuum distillation at pressures below 1 mm. Hg. The distillation apparatus consisted essentially of one 10-ml. distilling flask delivering into a second of like capacity. The latter was immersed in either a dry ice or cold water bath as needed. The course of the distillation was followed by measurement of refractive index with an Abbe refractometer. After considerable experience with the use of this apparatus and the type of material being distilled, it was found possible to obtain relatively pure yields of furfuryl alcohol and hydroxymethylfurfural. Where these two compounds were

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encountered in this study, their identification was accomplished according to procedures previously reported (5, 6).

*Simplified systems employing lactose.* The formation of furfuryl alcohol in lactose-casein systems, but not in those of lactose and glycine (5), suggested that some native property of casein might be modifying the lactose degradation. Of such properties, buffering capacity, the association of copper ions and the presence of basic groupings in the protein seemed worthy of consideration.

Accordingly, 1-kg. samples of 15 per cent lactose solution were prepared in combination with each of the following: glycine (30 g.), glycine (30 g.) plus copper sulfate (2 ppm. cupric ion), lysine hydrochloride (8 g.), lysine (6 g.), sodium bicarbonate (20 g.), 3 *N* HCl (sufficient to adjust pH to 2.5). The quantities of furfuryl alcohol and hydroxymethylfurfural recovered from the autoclaved samples are presented in table 1.

TABLE 1

*The amounts of certain furan compounds isolated from various heated lactose systems*

Systems <sup>a</sup> studied	Before heating	After heating	Neutral ether- extractable matter	Furfuryl alcohol	Hydroxy- methyl- furfural
	(pH)	(pH)	(g.)	(g.)	(g.)
Lactose + Glycine (30 g.)	7.0	4.1	0.41	None	0.38
Lactose + Glycine (30 g.) + Cu <sup>++</sup> (2 ppm.)	7.0	4.1	0.40	None	0.35
Lactose + Lysine HCl (8 g.)	4.6	3.6	0.45	None	0.40
Lactose + Lysine (6 g.)	8.9	4.2	0.91	0.25	0.24
Lactose + NaHCO <sub>3</sub> (20 g.)	8.3	4.8	2.80	0.87	Trace
Lactose + HCl	2.5	2.4	0.20	None	0.20

<sup>a</sup> 127°C. - 2.5 hr.

<sup>b</sup> 1-kg. quantities of 15% lactose solutions with the indicated material added.

*The effect of pH in heated skimmilk.* The data of table 1 indicate that pH is a vital factor affecting the formation of furfuryl alcohol and hydroxymethylfurfural from lactose. Those systems having basic initial pH produced significant quantities of furfuryl alcohol, whereas the acidic or neutral systems produced only hydroxymethylfurfural. Thus, it might be presumed that increasing the acidity of milk would favor the production of hydroxymethylfurfural and reduce the amount of furfuryl alcohol formed during heat treatment. This was observed to be the case. In demonstrating this point, 2-kg. samples of condensed skimmilk (30 per cent total solids) were used. One sample was adjusted to pH 4.8 with 3 *N* HCl; a second sample was retained unaltered (pH 6.4). Following autoclaving, 0.60 g. of hydroxymethylfurfural was recovered from the acidified sample. Furfuryl alcohol could not be isolated from this sample, although qualitative tests suggested that trace quantities of the compound might be present. The milk sample with pH unadjusted yielded 0.47 g. of furfuryl alcohol but no measurable quantity of hydroxymethylfurfural.

**Lactose- $\text{NaHCO}_3$  systems.** The relatively high yield of furfuryl alcohol obtained from the lactose- $\text{NaHCO}_3$  sample, as shown in table 1, appeared to be a significant finding and was investigated further. One and one-half kg. samples of 10 per cent lactose solution combined with varying amounts of  $\text{NaHCO}_3$  were autoclaved for 6 hr. at  $127^\circ \text{C}$ . One sample employing a  $\text{NaH}_2\text{PO}_4$ - $\text{NaOH}$  buffer (pH 6.5) was included also in the trial. Data were taken relative to changes in pH and the amounts of furfuryl alcohol formed in the samples during heating (table 2). These data demonstrate that buffer capacity of the lactose solution affects the yield of furfuryl alcohol. A change in pH to acidic conditions is beneficial to the yield; however, too rapid a shift to acidic conditions is apparently detrimental to the yield. This mechanism is considered in some detail under the discussion section.

TABLE 2

*The amounts of furfuryl alcohol formed and the changes in pH of heated<sup>a</sup> lactose solutions<sup>b</sup> containing varied amounts of  $\text{NaHCO}_3$*

Sample no.	$\text{NaHCO}_3$ added	Before heating	After heating	Furfuryl alcohol
	(g.)	(pH)	(pH)	(g.)
1	5	8.1	4.5	0.15
2	10	8.2	4.6	0.46
3	20	8.3	4.8	1.07
4	40	8.3	5.1	0.25
5	60	8.3	5.6	0.15
6	80	8.3	6.8	trace
7	*	6.5	5.2	0.22

<sup>a</sup>  $127^\circ \text{C}$ .-2.5 hr.

<sup>b</sup> 10% by weight.

\* 20 g.  $\text{NaH}_2\text{PO}_4$ ,  $\text{H}_2\text{O}$  added and pH adjusted to 6.5 with 3 N  $\text{NaOH}$ .

**Furan compounds as intermediates.** With respect to the mechanism of furfuryl alcohol formation, the possibility existed that some other furan compound might serve as a "precursor". Of such compounds, consideration was given to furfural and hydroxymethylfurfural. Although furfural should be readily recovered by the ether extraction technique employed, it was conspicuous by its absence in this and previous investigations (4, 5, 6). Conceivably, it could be reduced to furfuryl alcohol under the conditions of the reaction.

The addition of 3-g. quantities of furfural or hydroxymethylfurfural to 2-l. samples of skimmilk (9 per cent total solids) prior to heating did not increase the amounts of furfuryl alcohol produced during autoclaving. Ether extraction of these samples recovered 0.9 g. of furfural and 1.65 g. of hydroxymethylfurfural, respectively. The quantity of furfuryl alcohol recovered from both samples containing the added furan compounds, as well as from a control sample, was 0.1-0.2 g. It is evident, therefore, that the compounds considered do not serve as "precursors" of furfuryl alcohol in heated milk. They appear to undergo partial destruction during the heating treatment.

**Various sugars as sources of furfuryl alcohol.** It was noted previously that

glucose or galactose when heated in a casein solution gave rise to hydroxymethylfurfural but not furfuryl alcohol (5). Maltose, sucrose and methyl- $\alpha$ -D-glucopyranoside were studied in similar experiments. One-kg. samples containing 10 per cent of the sugars and 20 g. of  $\text{Na}_2\text{CO}_3$  were autoclaved for the 2.5-hr. period. It was noted that maltose produces furfuryl alcohol, but that sucrose and methyl- $\alpha$ -D-glucopyranoside do not.

*Control experiments.* Small quantities (3 g.) of furfuryl alcohol could be recovered to the extent of 95 per cent from aqueous solution (2 l.) with the ether extraction procedure and apparatus<sup>2</sup> used in these experiments. Recovery of hydroxymethylfurfural under these conditions was 93 per cent. No allowance is made in these recoveries for manipulative losses in weighing and drying, thus extraction of the compounds was approximately quantitative. The stability of pure lactose solutions to the heat treatment used in this study has been demonstrated previously (5).

#### DISCUSSION

The results of this study indicate that the formation of furfuryl alcohol from lactose is fundamentally a consideration in carbohydrate chemistry. Although furfuryl alcohol is one of the principal heat-generated compounds of milk or lactose-casein systems, findings herein show that the compound may be produced in pure lactose solutions having the required pH and buffer capacity (tables 1 and 2). With reference to milk, it appears that various protein groups and soluble salts create such conditions.

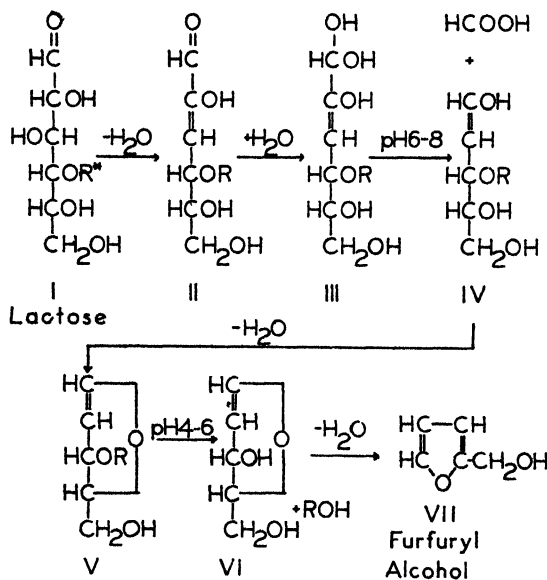
The data in table 1 clearly show a relationship between furfuryl alcohol and hydroxymethylfurfural. In both acidified skim milk and lactose systems, initial pH values below 6.0 appeared to favor the formation of hydroxymethylfurfural at the expense of furfuryl alcohol. This relationship was reversed under more alkaline conditions. Pigman and Goepp (7) state that sugars exhibit their maximum stability at acid conditions rather than at pH 7. Thus, it might be expected that heat degradation of lactose in milk (pH 6.6) would resemble lactose degradation under weakly alkaline conditions.

The formation of furfuryl alcohol from lactose or maltose but not from glucose, galactose or sucrose suggests that the disaccharide molecule with a 1,4 linkage between the hexose components is necessary in the parent compound. It seems logical, also, to assume that the glucose portion of lactose, having the hemiacetal configuration, would undergo degradation most readily and would, therefore, provide the carbon skeleton for furfuryl alcohol. This contention is supported by the general susceptibility of hemiacetals to chemical reaction and the observed stability of methyl- $\alpha$ -D-glucopyranoside under the experimental conditions employed.

The synthesis of furfuryl alcohol, a 5-carbon compound, from a glucose component containing 6 carbons raises a question as to how the extra carbon is eliminated. Evans *et al.* (1, 2) have theorized that the amount of formic acid produced by alkaline degradation of glucose or galactose is a partial indication of

<sup>2</sup> Ace Glass Co., Inc., Vineland, N. J.

cleavage at the 1,2 position in the hexose. They state further that a pentose residue should be the other resultant of the reaction. Insofar as is known, the demonstration of furfuryl alcohol as a degradation product of lactose constitutes the only direct evidence that such a pentose is formed. The work of Gould (3) has established the fact that formic acid is the principal volatile acid of heated milk. Whittier and Benton (9) have shown that the origin of such acids in heated milk is lactose. It is proposed that formic acid and furfuryl alcohol are related in the same mechanism of lactose degradation. This mechanism may be as follows (fig. 1):



\*Galactosyl

FIG. 1. A proposed mechanism for the chemical conversion of lactose to furfuryl alcohol.

In figure 1, the removal of a molecule of water between the 2,3 positions of lactose (I) is suggested by data from Wolfrom, *et al.* (10) on the degradation of glucose. The resulting enol II or its hydrate III decomposes at pH values above approximately 6 to yield formic acid and the structure IV. The definition of such pH requirements is indicated by the fact that at more acid reactions hydroxymethylfurfural is formed, which compound maintains the 6-carbon chain intact. Ring closure of IV is accomplished by removal of water. At pH values below approximately 6, galactose is hydrolyzed from V. The necessity of this pH condition is indicated by the data in table 2 which shows that a shift of pH from 8.3 to 6.8 accomplished little or no conversion of lactose to furfuryl alcohol. The removal of a final molecule of water from structure VI results in furfuryl alcohol VII.

If the enols II or III in figure 1 did not eliminate formic acid, as might be the case at pH values below 6, the reaction would be somewhat modified. Under these conditions the aldehyde group would remain intact and the end product of the reaction would be hydroxymethylfurfural. This latter reaction scheme is essentially a variation of that presented by Wolfrom *et al.* (10) for the conversion of glucose to hydroxymethylfurfural. An additional alternative may involve hydrolysis of lactose as the first step.

#### SUMMARY

The mechanism by which furfuryl alcohol and hydroxymethylfurfural are heat-generated in condensed skim milk and certain lactose systems has been studied. The importance of pH and buffer capacity in the reactions concerned has been demonstrated and discussed. Condensed skim milk and weakly alkaline lactose systems produced both furfuryl alcohol and hydroxymethylfurfural. Acidified condensed skim milk and neutral or acidic lactose systems yielded significant quantities of hydroxymethylfurfural but no furfuryl alcohol.

The structure of the lactose molecule or that of a similar sugar, maltose, was shown to be rather specifically required in furfuryl alcohol formation.

A proposed mechanism for the conversion of lactose to furfuryl alcohol has been presented schematically. In this mechanism the production of furfuryl alcohol is related to that of formic acid. It is suggested that a variation of the mechanism accounts for the conversion of lactose to hydroxymethylfurfural.

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# CHANGES IN WEIGHT OF THE REPRODUCTIVE ORGANS OF THE DAIRY COW AND THEIR RELATION TO LONG-TIME FEEDING INVESTIGATIONS

R. B. BECKER, P. T. DIX ARNOLD AND SIDNEY P. MARSHALL  
*Florida Agricultural Experiment Station, Gainesville*

Changes in body weights of dairy cows on long feeding trials involve more than changes in body fat. These weight changes may entail growth, gain or loss of fat, fetal development and alimentary contents. "Pasture Investigations Technique" (1) prepared in 1943, recommended (p. 358) that 3.53 lb. of total digestible nutrients be allowed in computations for each pound of gain and 2.73 lb. per pound of loss in body weight. When, and to what extent these weight changes occur, requires more exact consideration than given previously (1, 5, 11, 12). The Subcommittee on Animal Nutrition of the National Research Council (12) and Morrison (15) recommend requirements for body maintenance, milk production and cows advanced in gestation. The nutrient requirements recommended for cows in advanced gestation are liberal in allowing for body maintenance, milk production, if any, storage of reserve fat and mineral matter for the next lactation and the relatively low requirements for fetal development.

When attempts are made to regulate body weights of cows on long-time investigations by adjusting feed offerings, consideration should be given to that part of total body weight attributable to gestation, namely: changes in the uterus, placenta, embryo, accompanying fluids and slight ovarian changes.

## LITERATURE CITED

Eckles (4) attempted to measure nutrients required to develop the bovine fetus and found them too small to determine on a per day basis. He reported 3.9 lb. of dry matter in the amniotic fluid and placenta, while a 75 lb. Jersey calf contained 20.2 lb. of dry matter. He stated: "Four Jersey calves analysed at birth contained an average of 73.09 per cent of water. Data available indicate that breed is not a factor influencing the composition of new-born calves. The amniotic fluid weighs about thirty pounds and contains approximately 95 per cent water. The placenta weighs about 18 pounds, of which approximately 85 per cent is water.

"A Jersey cow produces a total of only fifteen or twenty, and a Holstein twenty or twenty-five pounds of dry matter in the fetus and its accompanying fluid and membranes. . . . The actual energy in the fetus and its accompanying fluid and membranes calculated from the weights and composition was 56.4 therms, a figure surprisingly close to the calculated requirement of 47.4 therms."

Yapp (19) found a maximum of 2.17 per cent of dry matter in the amniotic fluids from nine bovine fetuses and stated: "The highest total solids found for fetus and placenta (combined) was just over 24 per cent and the minimum, 2.56 per cent. Per cent ash varies from 0.82 in the youngest fetuses (41 days) to

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4.14 in the oldest (277 days); ether extract from only a trace to almost 4.0 per cent."

Non-pregnant uteruses analysed 21.7 per cent dry matter and 19.2 per cent protein, as compared with 16.8 and 13.5 per cent, respectively, in pregnant uteruses.

Haecker (6) and Hills (9) determined maintenance requirements of dry barren cows, from which the recommended requirements were liberalized conservatively by the Committee on Animal Nutrition (12) and Morrison (15). It is logical to assume, with Eckles (4) and Yapp (19), that nutrients required for combined development and maintenance of the highly moist bovine reproductive tissues may approach a level with those of the cow's more dense body.

Hayden (8) observed an average increase in body weights of 164 lb. with 426 Jersey cows before calving and a loss of 102 lb. at parturition, not allowing for involution of the uterus. It is logical to assume that a large part of the 62 lb. difference represented storage of body fat which may be available during the subsequent lactation period.

Putnam and Henderson (17) observed with 56 Ayrshires that the gains in body weight were not great before the fifth month of pregnancy. Under their conditions, "from 75 per cent to 85 per cent of the gain came in the last four months of pregnancy." They ascribed part of the weight increases to growth and calculated that this amounted to 27 per cent of the gains during the second gestation, and 20 per cent in the third pregnancy. These animals averaged 59 mo. of age at the third parturition.

With Holstein-Friesians, Moseley *et al.* (16) noted an average change from 1,435 lb. before calving, to 1,280 lb. on the next day, attributing 97 lb. to the calf and 58 lb. to placenta and fluids. This did not account for subsequent involution of the uterus.

Bartlett (2) analysed body weights of 59 milking Shorthorns and observed a 13.75 per cent increase in body weight attributable to pregnancy, based on the farrow weight after parturition. He estimated the loss of weight upon calving to be a 90-lb. calf and 80 lb. of placenta, amniotic fluids, etc.

Morgan and Davis (14) tabulated weight changes due to calving, growth and condition for 656 pregnancies in cows of four dairy breeds. The average decrease of Jersey cows from loss of fetus, membranes and fluids at calving amounted to 7.4 to 9.5 per cent of the cow's weight. Ayrshires, Guernseys and Holstein-Friesians did not vary widely beyond these percentages.

Swett *et al.* (18) analysed data on contents of 113 gravid bovine uteruses (55 from Beltsville and 58 from 13 cooperating state experiment stations). He found average weights of uteruses and contents (including vaginas) to range from 2.3 lb. at 14 days, to 148.5 lb. at 276 days in gestation. "After allowing for the weight of the nonpregnant uterus (3.57 pounds—average for combined breeds) these figures represent the portion of a cow's gains in live weight during pregnancy that are not attributable to changes in her condition (fatness)."

#### EXPERIMENTAL

As pregnant cows were discarded from the Florida Agricultural Experiment

Station dairy herd, weights were taken of the uteruses, fluids, placentae and fetuses. Non-gravid uteruses were weighed, as a base to compute increases due to pregnancy. Most of these cows were slaughtered and reproductive organs severed from the vagina posterior to the os uterus. The gravid uterus and ovaries were separated and weighed. Age of each fetus was computed from the date of service, even though Miller and associates (13) pointed out that conception may occur 1 to 3 days after service. Relation of length of gestation period to birth weights of Jersey calves in the station herd was determined.

Weights of 37 Jerseys, one Guernsey-Jersey, and one Guernsey embryo, together with weights of the placentae, fluids and uteruses, supply data applicable in interpreting weight changes of Jerseys. The combined weight of the ovaries and corpus luteum was between 10.7 and 22.5 g. and did not exceed weights of ovaries from non-pregnant cows. They are included in weights of "total uterus and contents."

The average weight of eight non-gravid uteruses was 1.4 lb. (see table 1). Gravid uteruses weighed up to 12.9 lb. at 7.5 to 8 mo. in gestation. About a 10 per cent increase in weight was observed beyond this time by Hammond (7), Bergmann (3) and one Florida observation.

TABLE 1  
*Weights of non-gravid uteruses from dairy cows*

Cow	Breed	Age	No. of gestations	Interval since calving	Net uterus <sup>b</sup>
		(yr. mo. d.)		(d.)	(lb.)
123-F	Jersey	4-7-19	barren	0	0.8
695-UF	Jersey	5-8-22	barren <sup>a</sup>	0	1.8
954-UF	Jersey	7-5-21	4	2	13.3 <sup>c</sup>
950-UF	Jersey	6-10-0	4	29	1.25 <sup>d</sup>
806-UF	Jersey	8-11-21	6	82	1.8 <sup>e</sup>
95-F	Jersey	3-8-7	2	98	0.95
601-UF	Jersey	12-0-11	9	104	1.85
107-F	Guernsey	4-2-28	2	289	1.6
906-UF	Jersey	7-6-16	5	505	1.17

<sup>a</sup> Used previously, as an uncertain breeder, in stilbestrol investigation.

<sup>b</sup> Uterus severed at the os uterus, and including the latter.

<sup>c</sup> Died within 54-58 hr. of milk fever and uterine complications after calving. This weight was not used in computations.

<sup>d</sup> In 950-UF, locations of 80 cotyledons were visible on inner uterine wall.

<sup>e</sup> Estrus occurred on day previous to slaughter; uterus was congested. Eight non-gravid uteruses average 1.4 lb. The separated vagina of 695-UF weighed 1.45 lb.; vulva 0.55 lb.

The chorion and amnion were not separated in most instances, consequently only total moist weights of fetal membranes (placenta) are given. Their increase during gestation was up to 7.6 lb. at 278 days. Eckles (4) observed weights of 10.5 to 18.5 (average of 15.8) lb. for three placentae from Jerseys at full term.

The amounts of fluids contained in the fetal membranes varied widely with individuals, being approximately 1.2 lb. or less prior to 2.5 mo. in gestation. A rapid gain occurred in the next 2 mo., with only a slight further increase until the last month of gestation. In a single instance, Eckles determined a total embryonic fluid weight of 32.7 lb. at nearly full term upon slaughter of the cow.

One Florida Jersey had 56 lb. of fetal fluids at 278 days. Hammond (7) commented concerning embryonic fluids as follows: "Whether the cessation of the increase in foetal fluids at the 5th month of pregnancy is connected in any way with the definite changes which occur in the udder at this time (in Shorthorn heifers) it is not possible to say without experimental evidence; but . . . an actual absorption of foetal fluids into the maternal circulation might supply a cause."

Individual Jersey fetuses varied in weight as birth weights of full-term calves also vary. These records (table 2) showed that a Jersey fetus attained a weight of about 1.0 lb. at about 3.5 mo. in gestation, that it weighed about 12 lb. at 6 mo. and that growth increases were rapid thereafter. Fetuses weighed over 40 lb. at 8 mo. in gestation. Average birth weights of 759 Jersey calves in the

TABLE 2  
*Changes in weights of uterus and contents during gestation*

Age of fetus	Age of dam	Uterus and contents	Fetus	Sex	Fetal fluids	Fetal membranes	Empty uterus
(d.)	(yr.)	(lb.)					(lb.)
31	14	4.3 <sup>a</sup>	0.27 g.	♀	3.32 g.	2.30 g.	4.3 <sup>a</sup>
31	5	1.11	0.28	♀	20.48	3.14	1.05
34	3	2.3 <sup>a</sup>	0.47	♀		6.42	2.0 <sup>a</sup>
35	6	1.47	0.51	♀	62.97	5.72	1.31
36	3	2.2 <sup>a</sup>	0.60	♀	28.8	3.11	1.48 <sup>a</sup>
39	4	1.5	1.01	♀		15.3	
44	6	1.09	1.70	M	63.67	17.71	0.85
44	5	1.27	1.72	M	87.50	10.20	1.06
56	7	6.1 <sup>a</sup>	10.64	M	453.0	70.0	4.92 <sup>a</sup>
59	3	2.61	11.94	M	419.28	125.0	1.23
72	9	3.8	43.74	M	720.5	142.0	1.75
74	6	3.1	44.0	F	546.0	101.0	1.5
77	5	2.6	56.66	M	707.0	100.0	0.69
89	6	4.7	172.5	M	1023.0	202.85	1.6
93	6	6.4	0.5 lb.	M	3.13 lb.	0.64 lb.	2.13
100	3	7.0	0.62	F	3.2	0.84	2.4
109	7	10.0	1.08	M	4.86	0.76	3.3
113 <sup>b</sup>	5	12.3	1.06	F	5.2	1.05	2.0
121	2	14.95	2.9	M	8.2	1.05	2.85
127	6	16.8	1.96	F	9.59	1.4	3.85
127	7	18.8	2.18	M	11.07	1.65	3.9
131	3	21.0 <sup>a</sup>	2.45	F	11.2	1.65	4.9 <sup>a</sup>
138	4	20.6	3.7	M	11.0	1.7	4.1
148	5	26.5	4.0	F	15.0	2.25	5.0
151	8	23.85	4.4	F	12.15	1.9	5.55
155	4	19.6	6.2	F	7.1	1.8	4.5
158	4	32.0 <sup>a</sup>	7.4	M	14.0	2.3	8.3 <sup>a</sup>
166	4	26.7	8.9	M	7.75	4.05	6.0
177	4	31.4	10.1	F	11.1	3.2	7.1
180	5	36.0 <sup>a</sup>	12.0	M	10.15	4.3	9.55 <sup>a</sup>
186	9	26.9	15.0	M	6.1	2.13	5.6
204	6	41.6	19.1	F	9.05	3.9	9.3
228	4	62.5	30.5	F	13.0	5.5	13.5
231	4	64.85	33.0	M	12.15	5.35	11.35
235	7	58.6	28.0	M	14.3	5.0	11.3
236	3	71.5	42.0	F	10.9	6.0	12.6
236	3	75.45	45.0	M	14.5	(15.95)	
245	3	77.6	41.0	M	17.1	6.6	12.9
278	3	140.0	61.45	M	56.35	7.6	14.6

<sup>a</sup> Combined weight, including the vagina.

<sup>b</sup> Guernsey.

Florida station herd over an 18-yr. period were determined. The 392 males ranged between 27 and 80 lb., averaging 55 lb., whereas 367 heifer calves ranged from 27 to 76 lb., the average being 52 lb. They tended to weigh less following short gestation periods.

Total weights of 39 uteruses and contents (all but seven weights excluded the vagina) ranged between that of non-gravid uteruses (1.4 lb.) and 140 lb. at 278 days in gestation. The latter contained a 61-lb. male fetus.

Increases of body weights due to gestation are calculated in table 3. Naturally, there would be no allowance or gestation in the case of an unbred cow. Also, the increase in weight of the uterus and contents during the first 60 days of gestation is negligible. The weight increase is estimated to amount to 5 lb. at 90 days in gestation, and increases to about 122 lb. at full term. This includes weight changes due to the fetus, placenta, fluids and involution of the uterus within 2 wk. after calving.

At parturition the uterus of the full-term cow weighed 14.6 lb., whereas involution reduced the non-gravid uteruses to an average of 1.4 lb. Applying Yapp's percentages for dry matter and protein to these weights leaves an unaccounted reduction of uterine tissue by 2.15 lb. of dry matter (1.59 lb. of protein).

The period immediately following calving is one of physiological underfeeding, ordinarily. It is presumed that this small nutrient difference may leave the cow's body during involution over a period of several days, whereas the fetus, placenta and fluid leave the cow's body shortly.

The 280-day gestation-weight estimate was combined from (a) average birth weight of 392 male Jersey calves in Florida, (b) three placentae reported by Eckles (4), (c) the difference between the combined weights of placentae and fluids (14) and the three placentae (4), and (d) the net empty uterus weight with the 278-day Jersey male fetus.

Morrison's maintenance standard (15) is regarded as sufficiently liberal to

TABLE 3

*Weight estimates of uteruses and contents and suggested allowances in body weight for stage of gestation with Jerseys*

Age of fetus	Reproductive organs and contents	Fetus	Membranes	Fluids	Net empty uterus	Weight increase due to gestation
(d.)	(lb.)				(lb.)	(lb.)
0	1.4	0	0	0	1.4	0
30	2.5	0.25 g.	3.1 g.	11.2 g.	2.4	negligible
60	2.6	12.0 g.	125.0 g.	0.9 lb.	1.4	1
90	6.4	0.5 lb.	0.6 lb.	3.1 lb.	2.2	5
120	14.0	1.7 lb.	1.0 lb.	8.1 lb.	3.2	12
150	23.0	4.3 lb.	1.9 lb.	11.5 lb.	5.3	22
180	32.0	11.1 lb.	3.2 lb.	10.6 lb.	7.1	31
210	44.5	19.5 lb.	4.4 lb.	11.1 lb.	9.5	43
240	76.0	40.0 lb.	6.3 lb.	16.7 lb.	13.0	75
270	111.7	51.3 lb.	13.5 lb.	32.6 lb.	14.2	110
280 ±	123.6	55.0 lb.	15.8 lb.	38.2 lb.	14.6 <sup>a</sup>	122 <sup>a</sup>

<sup>a</sup> The immediate drop in body weight at calving in this instance would be 109 lb., with an expected further reduction of 13.2 lb. (14.6-1.4) within 2 wk. due to involution of the uterus.

provide for actual maintenance of the cow's net body, as well as for reproduction. Greater increases than indicated in table 3 for gestation would be considered gains in actual body weight. Less than those amounts would be regarded as weight losses. They would be computed at 3.53 and 2.73 lb. of total digestible nutrients, respectively, and either credited or debited to the nutrients involved in respective total digestible nutrients computations.

Two methods of computing nutrients involved in analyses of feeding results were compared. Since no appreciable change occurred due to pregnancy during the first 60 days of gestation, the period of weight corrections would be the remaining 220 days of the gestation period. An 800-lb. cow will be used to illustrate application of weight corrections for gestation.

If maintenance requirements were computed daily by the Morrison standard on the basis of gross body weights (800 to 922 lb.), the accumulative total would amount to 1,551.97 lb. of total digestible nutrients. This does not evaluate the 122 lb. at the rate of 3.53 lb. of total digestible nutrients per pound of gain.

On the other hand, if maintenance needs had been computed in the same manner, and gains credited at 3.53 lb. of total digestible nutrients per pound for the 122-lb. gain due to pregnancy, the computation would have made it appear that an additional 430.66 lb. of total digestible nutrients had been received from the feed. This entails above a 27 per cent error from an over-evaluation. Converted to practical terms, 430 lb. of total digestible nutrients in 220 days would equal about 860 lb. of hay, or 3,071 lb. of fresh Napier grass leaves (10) or similar pasture grass. This error is appreciable either in technical terms or in practical interpretation of research results.

In view of the above, it is suggested that corrections for stage of gestation listed in table 3 be allowed before applying the factors of 3.53 and 2.73 to the gains or losses, respectively, in gross body weights of pregnant Jersey cows in long feeding trials. Corresponding factors need to be developed for other dairy breeds. Any attempts to regulate body weights by limiting feed allowances, should make due allowance for advancing stage of gestation during extended feeding trials.

#### SUMMARY AND CONCLUSIONS

Weights of nine non-gravid and 39 gravid uteruses and contents are presented, based on dairy cows slaughtered in the station herd. Significant changes in gross body weight occurred between the 61st day of gestation and full term. Division of these weight changes was approximated for Jersey cows at monthly intervals until ready to calve.

Using an 800-lb. cow as an example, computation of total digestible nutrient requirements by Morrison's standard, with 122-lb. gains evaluated at 3.53 lb. of total digestible nutrients per pound of gain, would amount to 1,983 lb. of total digestible nutrients. On the other hand, if growth and maintenance of the more highly moist fetus and associated tissues were attained within the liberal Morrison standard, an excess of over 430 lb. of total digestible nutrients would have been computed—a difference of over 27 per cent of maintenance would have been incurred over the last 220 days of gestation.

It is suggested that gross body weights be computed to net weights (less gains due to gestation) when trying to control body weights by regulating the level of concentrates fed during long feeding trials.

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## A COMPARISON OF THE ALLEN VOLUMETRIC BLOOD FAT PROCEDURE WITH AN EXTRACTION PROCEDURE<sup>1, 2</sup>

A. C. CHUNG<sup>3</sup>, P. SAARINEN AND J. C. SHAW

*Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park*

The method proposed by Allen (1, 2) for the determination of fat in blood plasma is easily and quickly carried out and permits the rapid determination of large numbers of samples. This method has been used to advantage in several studies (2, 3, 9, 10), but unfortunately the method has not been subjected to a critical study. Allen (2) made a comparison between Bloor's method for total fat and his own method on three samples of plasma and obtained values by his method, which were 36.8, 68.9 and 70.9 per cent of the total lipids obtained by Bloor's procedure. Based on the work of Petersen and Herreid (4) who developed the reagent for the determination of fat in buttermilk, it was concluded that the difference was due to the absence of phospholipids in the fat separated from plasma by the reagent.

No actual fractionations of this lipid column or comparisons with different plasma lipid fractions have been reported. Such comparisons are presented in this report.

### EXPERIMENTAL PROCEDURE

Four cows, two Guernseys and two Holsteins, were used for this study. Blood samples were taken from the jugular vein at weekly intervals. Potassium oxalate was used for an anti-coagulant and NaF was used as a preservative. The extractions were made immediately after centrifuging and the same samples were analyzed by the modified Allen procedure the following day.

Three different approaches were used in this investigation. First, the composition of the Allen fat column was determined. Secondly, the fractionations made on the Allen fat column were compared to the values obtained by the direct extraction of the same plasma. Lastly, a statistical study was made of the relationships between the Allen lipid values and the total plasma lipids and lipid fractions. Twenty-seven samples were analyzed for this part of the study.

A modification of the Allen volumetric procedure used for routine analysis in this laboratory was used in this study. For greater accuracy, the length of the fat column was measured through a glass window in a constant temperature bath by means of a reading microscope mounted on a micrometer slide as reported by Shaw (8). In addition, precision-bore capillary tubes with a diameter of 1.016 mm. were used in making the fat tubes so that the calibration factor was

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<sup>3</sup> The experimental data in this paper are taken in part from a thesis presented by A. C. Chung in partial fulfillment of the requirements for the degree of Master of Science in Dairy Husbandry, University of Maryland.

practically identical for all tubes. An additional small, but very helpful, improvement was made in the course of this study. Perhaps the most troublesome part of this procedure has been the necessity of inserting a small wooden plug in the top of the capillary tube before placing the tube in the water bath. This frequently resulted in the moving and breaking of the fat column so that this reading became less accurate. This was solved by not flaring the top of the capillary tube and placing a small piece of adhesive tape over the end of the tube instead of inserting wood plugs.

For the extraction and fractionation of the plasma lipids, Saarinen's method was used (5, 6, 7). Of the two alternatives proposed in the latter paper for the extraction of the total lipids, the more accurate extraction with alcohol-ether (2:1) was used in this study. The total lipids were saponified with a saturated aqueous solution of NaOH using reflux condensers. The phospholipid fatty acids values determined by the difference between the total lipids and the lipids other than phospholipids were converted to total phospholipid values, using the factor 1.44.

TABLE 1  
*The percentage composition of the Allen fat column*

Sample no. <sup>a</sup>	Total cholesterol	Ester cholesterol	Free cholesterol	Fatty acids in cholesterol esters	Neutral fat	Phospholipids
	(%)	(%)	(%)	(%)	(%)	(%)
1	54.6	47.0	7.5	30.5	11.5	3.4
2	59.5	51.4	8.1	31.8	4.0	4.6
5	55.6	46.0	15.6	30.6	12.8	1.0
9	56.1	46.8	10.7	31.0	12.4	0.4
10	55.8	44.3	11.5	29.4	14.6	0.2
Av.	56.3	47.1	10.8	30.7	11.1	1.9

<sup>a</sup> No. 1 and 2 represent mixed plasma samples, and no. 5, 9, and 10, single cows.

## RESULTS

The percentage composition of the fat column obtained by the volumetric method was determined on two mixed plasma samples and on individual samples from three other cows. The results are presented in table 1. In this case, the phospholipids were calculated on the basis of the total phosphorus in the purified fat. These data show that the fat column contained small but variable amounts of phospholipids. The main part of the lipids consisted of cholesterol esters, free cholesterol and neutral fat. The proportions of these fractions varied within the normal range, which indicated that most of the lipids were liberated by the volumetric procedure. The uniform composition of the different fat samples undoubtedly is due to the fact that the cows were fed similarly.

For purposes of comparison, four of the above plasma samples also were subjected to the complete fractionation procedure of Saarinen. The values obtained in the analysis of the lipids separated from these same samples by the Allen procedure were calculated back to the actual level in the blood plasma on the basis of the Allen total lipid value. The results of this comparison are shown in table 2. The data show that the lipid fraction consisting of cholesterol and cholesterol



TABLE 2

*A comparison of lipid fractions (Mg./100 ml. plasma) separated from plasma by the Allen and Saarinen procedures*

	Sample 1		Sample 5		Sample 9		Sample 10		Average	
	A <sup>a</sup>	S <sup>b</sup>	A	S	A	S	A	S	A	S
Total cholesterol (mg./100 ml.)	116.9	152.7	94.0	99.4	119.4	128.2	130.3	129.1	115.2	127.4
Ester cholesterol (mg./100 ml.)	100.6	118.3	77.7	72.9	99.6	105.1	103.4	110.2	95.4	101.6
Free cholesterol (mg./100 ml.)	16.3	34.4	26.3	16.5	19.8	23.1	26.9	18.9	19.8	23.2
Fatty acids in cholesterol esters (mg./100 ml.)	65.3	65.6	51.7	48.4	66.0	69.8	68.6	73.2	62.9	64.3
Neutral fat (mg./100 ml.)	24.6	0.8	21.6	43.3	26.5	28.5	34.1	29.3	26.7	27.3
Phospholipids (mg./100 ml.)	7.3		16.9		12.8		0.5		10.5	

<sup>a</sup> A = Allen procedure

<sup>b</sup> S = Saarinen procedure

and glycerol esters was removed almost as completely by the Allen volumetric procedure as by the Saarinen extraction procedure. In addition to the above lipid fractions, the Allen procedure included small amounts of phospholipids.

A summary of the data on 27 samples which were subjected to the Allen procedure and simultaneously fractionated more completely by the Saarinen procedure is presented in table 3. The mean value obtained by the Allen procedure was  $63.3 \pm 1.17$  per cent of the total lipids determined by the extraction procedure. The Allen lipid value was very similar to the value obtained for the lipids other than the phospholipids. The latter fraction averaged  $83.1 \pm 1.41$  per cent of the value obtained by Allen's procedure. This indicates that some lipids of phospholipid origin were included in the Allen fat column.

It will be noticed that rather high correlations were obtained between the Allen value and total lipids, lipids other than phospholipids and total cholesterol. Since only small amounts of phospholipids are separated by the Allen procedure,

TABLE 3

*The average (M) of different lipid fractions with standard errors ( $m_x$ ) and the correlations of other fractions to Allen's lipid values ( $n=27$ )*

Lipid fraction	Plasma lipids $M \pm m_x$ (mg./100 ml.)	Correlation to Allen's lipid value $r \pm m_r$
Allen's lipid value	$258.1 \pm 10.3$	
Total lipids	$530.9 \pm 17.4$	$0.866 \pm 0.048$
Phospholipids	$301.7 \pm 14.6$	$0.867 \pm 0.107$
Total cholesterol	$127.8 \pm 5.3$	$0.846 \pm 0.055$
Ester cholesterol	$110.9 \pm 5.1$	$0.803 \pm 0.068$
Free cholesterol	$16.9 \pm 1.6$	$0.846 \pm 0.169$
Total lipids minus p-lipids	$229.2 \pm 7.0$	$0.968 \pm 0.047$
Fatty acids in cholesterol and glycerol ester fraction	$101.4 \pm 8.7$	$0.408 \pm 0.161$

it is probable that the high correlation between the Allen value and the total lipids is accidental due to the limited data. Additional data are needed to establish these relationships more precisely.

As

#### CONCLUSIONS

The fractionation of the Allen lipid column showed that only small amounts of phospholipids were present. A comparison of these fractions with fractions obtained by extraction of the same plasma gave similar values, indicating that the lipids other than phospholipids are separated rather completely by the Allen procedure. The lipids other than phospholipids were  $83.1 \pm 1.41$  per cent of the Allen lipid value. The latter was  $63.3 \pm 1.17$  per cent of the total plasma lipids. The correlation between the Allen lipid value and the value for plasma lipids other than phospholipids was high ( $r = 0.866 \pm 0.047$ ).

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# THE VALIDITY OF THE ALLEN VOLUMETRIC PROCEDURE FOR THE DETERMINATION OF BLOOD LIPIDS OF COWS ON DIFFERENT FEEDING REGIMES<sup>1 2</sup>

H. K. LO,<sup>2</sup> P. SAARINEN AND J. C. SHAW

*Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park*

In a previous paper by Chung *et al.* (1), a comparison was made between a modified Allen blood fat procedure and a fractionation procedure based on the extraction of blood lipids. This study indicated that the Allen procedure was satisfactory under normal conditions for the determination of the plasma lipid fraction composed of the lipids other than phospholipids. However, it was deemed advisable to obtain additional data to establish the relationships more specifically and especially to determine the value of the procedure under varying feeding regimes. This report deals with the validity of a modification of the Allen volumetric method for the determination of blood lipids of cows on different levels of energy and fat intake.

## EXPERIMENTAL PROCEDURE

For this study, blood samples were drawn from cows which had been fed on different levels of fat and energy intake in connection with another project which was under way simultaneously. These rations resulted in marked variations in the plasma lipids. A group of 12 cows, seven Holsteins, two Guernseys and three Ayrshires, was used for this study. Two to three blood samples were drawn from each cow 14 to 18 days after each change in feeding. The blood was drawn from either the coccygeal artery by a method developed by Saarinen (2) or from the jugular vein. A total of 155 blood samples was analyzed.

All blood samples for this experiment were drawn between 8 and 10 a. m. during the last part of each period. Potassium oxalate was used as an anti-coagulant and about 0.1 per cent of NaF was used as a preservative. The plasma was analyzed immediately after centrifuging, except in one case when the blood samples were stored in a refrigerator 1 day.

The analytical methods were the same as those used in the previous paper (1), except that the total lipids were not saponified in the first set of samples. Consequently, the phospholipids were determined directly as true phospholipids.

## RESULTS

During the various test periods the plasma total lipids varied from 213.3 to 623.3 mg. per cent. The plasma phospholipids paralleled the total lipids closely

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<sup>3</sup> The experimental data in this paper are taken in part from a thesis presented by H. K. Lo in partial fulfillment of the requirement for the degree of Master of Science in Dairy Husbandry, University of Maryland.

TABLE 1

*The relationships between the Allen volumetric plasma lipid value and different plasma lipid fractions obtained by Saarinen's procedure*

Lipid fraction	M $\pm$ m <sub>M</sub>	Correlations to Allen lipid value r $\pm$ m <sub>r</sub>
	(mg./100 ml.)	
Allen lipid value	220.4 $\pm$ 4.3	
Total lipids	375.4 $\pm$ 6.7	0.663 $\pm$ 0.045
Phospholipids	152.2 $\pm$ 5.0	0.109 $\pm$ 0.079
Total cholesterol	151.5 $\pm$ 2.7	0.766 $\pm$ 0.033
Ester cholesterol	103.9 $\pm$ 2.1	0.750 $\pm$ 0.035
Free cholesterol	47.6 $\pm$ 1.4	0.382 $\pm$ 0.069
Total lipids minus phospholipids	223.2 $\pm$ 3.5	0.836 $\pm$ 0.024
Fatty acids in the cholesterol glycerol ester fraction	71.7 $\pm$ 1.7	0.550 $\pm$ 0.056

as has been observed previously by Saarinen (3). The other lipid fractions, including the volumetric value, varied more independently of the total lipid values. The mean volumetric value was 58.6 per cent of the mean total lipid value and 98.5 per cent of the value for lipids other than phospholipids.

The correlations to the volumetric lipid value were calculated separately for each plasma lipid fraction. The coefficients of correlations along with the means and standard errors are shown in table 1. Not only was the mean value obtained by the volumetric procedure ( $219.81 \pm 4.29$ ) very similar to the mean value for the lipid fraction other than phospholipids ( $223.19 \pm 3.53$ ), but the coefficient of correlation between these two values was relatively high ( $0.836 \pm 0.024$ ). The coefficient of correlation between the volumetric value and the plasma phospholipids was very low ( $0.109 \pm 0.079$ ). This was to be expected due to the large variations in plasma lipids produced by variations in feeding. The other correlations are about what would be expected on the basis of the percentage representation of these fractions in the lipids other than phospholipids.

These data establish the fact that the volumetric procedure is fairly well adapted to the determination of lipids other than phospholipids under markedly different feeding conditions.

TABLE 2

*Correlation between true total lipids minus the Allen lipid value and the Saarinen phospholipid fraction*

	Present data (n - 155)		Chung's data (n - 27)	
	M $\pm$ m <sub>M</sub>	Correlation to p-lipids obtained by Saarinen method r $\pm$ m <sub>r</sub>	M $\pm$ m <sub>M</sub>	Correlation to p-lipids obtained by Saarinen method r $\pm$ m <sub>r</sub>
	(mg./100 ml.)		(mg./100 ml.)	
Phospholipids obtained by the Saarinen method	152.2 $\pm$ 5.0	.. ..	301.7 $\pm$ 14.6	.. ..
Total lipids minus the Allen lipid value	155.0 $\pm$ 5.6	0.905 $\pm$ 0.015	272.8 $\pm$ 10.9	0.780 $\pm$ 0.181

The above fact indicated that it would be possible to determine the plasma phospholipids by the difference between the total lipids and the Allen volumetric lipid value. To test this hypothesis, coefficients of correlations between the true phospholipid fraction and the total lipids minus the volumetric lipid value were calculated from both the present data and from the data by Chung *et al.* (1). These results, along with the means and standard errors, are presented in table 2. The coefficients of correlation were  $0.905 \pm 0.015$  and  $0.817 \pm 0.065$ , respectively, which suggests that this procedure may be followed in estimating plasma phospholipid values.

#### SUMMARY AND CONCLUSIONS

Determinations of blood plasma lipids were made on 155 blood samples from 12 cows on markedly different feeding regimes. All of the samples were subjected to analysis by an extraction procedure and by a modification of the Allen volumetric procedure.

A relatively high coefficient of correlation ( $0.836 \pm 0.024$ ) was found between the mean volumetric value and the lipid fraction consisting of the total lipids other than phospholipids, which shows that the former method is fairly reliable for the determination of this lipid fraction.

On 155 samples a high coefficient of correlation ( $0.905 \pm 0.015$ ) between the true phospholipid value and the difference between the total lipids and the Allen volumetric lipid value suggests that phospholipids can be estimated fairly well merely by determining the volumetric lipid value and the total lipid value.

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## USE OF PROPYL GALLATE TO DEFER DEVELOPMENT OF OXIDIZED FLAVOR IN MARKET MILK<sup>1</sup>

W. H. CHILSON, W. H. MARTIN, AND C. H. WHITNAH  
*Kansas Agricultural Experiment Station, Manhattan*

One of the most difficult off-flavors to control in market milk is the oxidized flavor which may develop after a few days storage. This off-flavor is especially troublesome during the winter period.

Chilson *et al.* (2) and others (1, 4) have shown that the addition of ascorbic acid to market milk will defer the development of oxidized flavor for several days, but when the ascorbic acid is depleted, the off-flavor develops. Ascorbic acid is depleted quickly in the presence of added copper, and a more pronounced oxidized flavor develops than would have developed had not ascorbic acid been added (2). Such uncertain control measures may be somewhat unsatisfactory in many commercial milk plants. If a simple and practical treatment could be devised that would eliminate oxidized flavor for at least 1 wk. of storage, such a treatment would be accepted enthusiastically by milk processors.

Propyl gallate has been recommended as an antioxidant to be used in butter for candy manufacture (5). It was effective in controlling oxidized flavor in dried whole milk and dried ice cream mix (6). Propyl gallate is an approved ingredient in stabilized lard and lard and vegetable fat mixtures, the maximum content allowed being 0.01 per cent (5).

The research herein reported was undertaken to determine the effectiveness of propyl gallate in controlling the development of oxidized flavor in market milk.

### METHODS

Six trials were conducted, each consisting of split samples with the following treatment: 1. Control milk; 2. control milk plus 20 mg. of propyl gallate per liter of milk; 3. control milk plus 20 mg. propyl gallate per liter with 0.5 ppm. of added copper; 4. control milk plus 30 mg. of ascorbic acid per liter and 0.5 ppm. of added copper; 5. control milk plus 0.5 ppm. of added copper; 6. control milk plus 20 mg. of propyl gallate per liter plus 30 mg. of ascorbic acid per liter plus 0.5 ppm. of added copper. The 500-ml. samples used in each trial were taken from freshly pasteurized milk (holder process) from the college herd. Treatments were begun immediately after the samples were obtained. The propyl gallate was dissolved in glycerine before being added to the milk. The copper was added as an aqueous solution of  $\text{CuSO}_4$ . The ascorbic acid was added as a powder and thoroughly mixed with the milk by shaking. The samples were placed in quart milk bottles and then stored in a cabinet refrigerator at approximately 35° F. All samples were examined for the presence of oxidized flavor when fresh and after 1, 2, 3, 5, 7 and 14 days of storage. Samples were scored 40

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points if no oxidized flavor was evident. A score of 30 was used to denote a very pronounced oxidized flavor. Thus, scores from 40 to 30 denote the intensity of the off-flavor. Selected samples were analyzed chemically for ascorbic acid content and the oxidation-reduction potential determined.

### RESULTS

Since the flavor scores on corresponding samples of the six trials were nearly identical, varying only slightly in the intensity of the oxidized flavor on a particular day, the results are shown as average scores in table 1. After one day of

TABLE 1

*The effect of propyl gallate upon the development of oxidized flavor in market milk (average of 6 trials)*

Treatment	Days stored						
	Fresh	1	2	3	5	7	14
			Average flavor scores <sup>a</sup>				
Control—none	40	39.0	37.6	36.0	33.5	32.3	30
Control plus 20 mg. propyl gallate/l.	40	40	40	40	40	40	40
Control plus 20 mg. propyl gallate/l. plus 0.5 ppm. of Cu.	40	40	40	40	40	40	40
Control plus 30 mg. ascorbic acid/l. plus 0.5 ppm. of Cu.	40	35	33	30	30	30	30
Control plus 0.5 ppm. of Cu.	40	36	33.3	33	31.7	31.3	30
Control plus 0.5 ppm. of Cu. plus 30 mg. ascorbic acid plus 20 mg. propyl gallate/l.	40	40	40	40	40	40	40

<sup>a</sup> The milk used usually had a slight cooked or feed flavor, but a score of 40 was used to designate the absence of an oxidized flavor at the beginning of the trial. Scores from 40 to 30 indicate the degree of oxidized flavor.

storage, two of the six control samples were slightly oxidized, giving an average score of 39 for the six control samples. After 2 days in storage four of the control samples were oxidized, and by the fifth day all were distinctly oxidized, having an average score of 33.5.

The samples that contained added ascorbic acid and copper usually developed the most pronounced oxidized flavor in the shortest storage time, while those containing only copper were second. The control samples developed the least intense oxidized flavor of the samples not protected with propyl gallate.

All 18 samples that did not contain propyl gallate developed a definite oxidized flavor. Those containing propyl gallate with or without added copper developed no oxidized flavor within 14 days. The oxidized flavor was scored according to its intensity and objectionableness, regardless of the type. The control samples seemed to have more of a cardboardy or papery flavor, while those containing added copper were usually fishy, or tallowy and fishy, and those samples containing added ascorbic acid and copper usually were tallowy, or cardboardy and tallowy.

In four of the six trials, addition of propyl gallate to the control milk lowered the oxidation reduction potential an average of 0.059 volts. The initial potentials were from +0.165 to +0.280 volts. The greater reductions usually occurred on

samples having the higher initial potential. This is in agreement with the results of other investigations on oxidized flavor, which have demonstrated that a substance or process which controls or retards oxidized flavor also reduces the oxidation-reduction potential, at least temporarily (4).

Analyses of six fresh and four stored samples for ascorbic acid content showed that the addition of propyl gallate had practically no effect on the oxidation of ascorbic acid. The amount of ascorbic acid in five fresh samples to which propyl gallate was added was the same as the amount in the control samples. One fresh sample containing propyl gallate had 3 mg. or 17 per cent less ascorbic acid than the control milk. Analyses of three stored samples containing propyl gallate showed that they contained the same amount of ascorbic acid as the corresponding controls, and one stored sample containing propyl gallate showed a loss of 1.3 mg. or 33 per cent, as compared to the control.

#### DISCUSSION

Greenbank (3) and others (4) have shown a relationship between the oxidation-reduction potential and the development of oxidized flavor in milk. At least a part of the flavor-protective action of added ascorbic acid has been attributed to the fact that the oxidation-reduction potential is lowered. Since propyl gallate does not stabilize ascorbic acid, yet prevents the development of the oxidized flavor in milk even in the presence of added copper, it would seem that there is a difference in the nature of the protective actions afforded by these two substances. More information regarding the mechanisms involved in the protective actions of these substances would be interesting, but is beyond the scope of this paper.

It was intended that this study should include work to ascertain the minimum amount of propyl gallate that would protect milk from oxidation for a storage period of 14 days. However, by the time this phase was started, the college herd had been on spring pasture for several weeks and the milk no longer developed an oxidized flavor spontaneously, and even with added copper only a mild oxidized flavor developed after several days' storage.

Twenty milligrams of propyl gallate per liter was selected as the amount to use for this experiment, based on the amount used in dry whole milk by research workers of the Quartermaster Food Institute (6). A few limited trials indicated that 10 mg. of propyl gallate per liter of milk were effective in protecting against oxidized flavor development for a period of 7 days, and as little as 1.25 mg. per liter gave some protection. It seems reasonable to believe that substantially less than 20 mg. per liter should give ample protection to milk stored and distributed under normal commercial conditions.

It should be emphasized that propyl gallate may be classed as a drug. It is not a food product. Therefore, the addition of this product to milk or milk products for other than experimental purposes might bring prosecution, if not approved by enforcement officials.

#### CONCLUSION

The addition of propyl gallate to freshly pasteurized milk at the rate of 20



mg. per liter was found to prevent the development of oxidized flavor effectively for a 14-day storage period at 35° F. The propyl gallate treatment was equally effective with or without 0.5 ppm. of added copper.

The ascorbic acid, natural or added, was not stabilized by the propyl gallate.

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# WHITE MUTANTS OF *PENICILLIUM ROQUEFORTI*<sup>1</sup>

S. G. KNIGHT, W. H. MOHR AND W. C. FRAZIER

*Department of Agricultural Bacteriology, University of Wisconsin, Madison*

Induced mutation of microorganisms has become a useful tool in microbiological research. Lederberg (3) cites many instances where studies of mutants have contributed to our knowledge of microbial genetics and physiology. From a practical point of view, Backus *et al.* (1) have produced by ultraviolet irradiation mutants of *Penicillium chrysogenum* that yield more penicillin than the parent. This is a preliminary report of a series of studies on normal and mutant strains of *Penicillium roqueforti*. These studies were undertaken for the purpose of obtaining information about the genetics and physiology of *P. roqueforti* in the hope that such information might be of value in the manufacture of mold-ripened cheese.

## METHODS

Two molds designated as *P. roqueforti*, strains 1 and 2, were obtained from the Division of Dairy Husbandry of the University of Minnesota. These strains, as well as the mutants therefrom, were grown on a medium made by mixing equal parts of sterile "V8" vegetable juice and sterile 6 per cent agar.

Mutants were obtained by ultraviolet irradiation of spores that had been spread on the surface of vegetable juice agar in a petri dish. The petri dishes were placed 18 cm. from a Westinghouse "Sterilamp," and the inoculated surface of the medium was irradiated for about 20 min., a treatment which killed almost all of the spores on the medium. After irradiation the plates were incubated at 25° C. Many of the molds that grew after incubation of the plates were mutants, but only the mutants that produced white spores were picked for further study. Spontaneous mutation of the green molds to white molds never was observed.

To study the lipolytic and proteolytic activity, the parent and mutant strains were grown on sterile milk that had been adjusted to 8 per cent butterfat. Lipolytic activity was estimated by the amount of volatile, water-soluble acid produced from 100 ml. of milk fortified with fat. Proteolytic activity was estimated from the amino nitrogen produced, as assayed by the Van Slyke procedure.

## RESULTS

After ultraviolet irradiation many of the surviving spores formed colonies of molds that unquestionably were mutants. The mutants most frequently found were slow-growing types. Mutants that were nonsporulating or had oddly formed hyphae were observed occasionally. Mutants that formed white spores

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but otherwise were normal were uncommon. Nevertheless, ten stable white-spore mutants were obtained and four of them were used in this study. White mutants 1-5 and 1-10 were obtained from *P. roqueforti*, strain 1, and white mutants

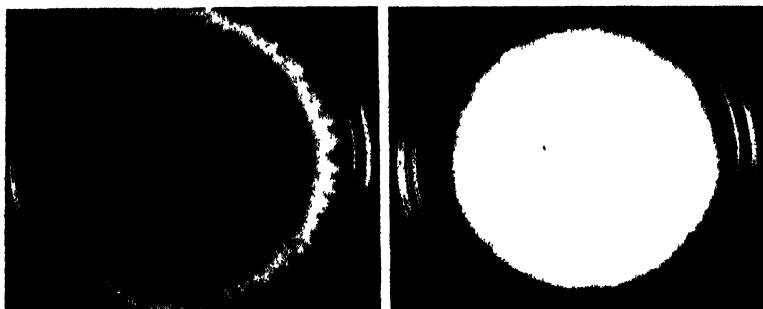


FIG. 1. Normal *P. roqueforti* (strain 1) and a typical white mutant (strain 1-10).

2-1 and 2-10 were obtained from strain 2. Figure 1 is a photograph of a normal *P. roqueforti* and a typical white mutant. The white mutants apparently are stable, since all attempts to induce reversion to green spores have been unsuccessful.

TABLE 1

The ml. of *N*/0.05 KOH necessary to titrate the soluble volatile acid produced by the lipolytic activity of the normal and white *P. roqueforti* on 100 ml. milk plus 8% butterfat at 25° C

Strain	ml. of <i>N</i> /0.05 KOH		
	(days of incubation)		
	5	9	15
green (1)	6.1	6.7	8.5
white (1-10)	6.3	7.3	17.2
white (1-5)	5.3	6.0	8.2
green (2)	6.5	7.4	11.7
white (2-10)	7.8	11.5	17.5
white (2-1)	6.8	8.0	10.3

Furthermore, when mixtures of various combinations and proportions of spores from ten white mutants were mixed and plated in an attempt to produce colored heterocaryons, only colonies with white spores were formed.

TABLE 2

The amino-N produced by the proteolytic activity of normal and white *P. roqueforti* on milk plus 8% butterfat at 25° C

Strain	mg. of amino-N/ml.		
	(days of incubation)		
	5	9	15
green (1)	0.57	1.05	1.22
white (1-10)	0.46	1.05	1.28
white (1-5)	0.45	1.01	1.25
green (2)	0.45	0.86	1.08
white (2-10)	0.34	0.96	1.48
white (2-1)	0.46	1.07	1.08

A comparison of the lipolytic activity of the normal molds and the white mutants is shown in table 1. Apparently lipolytic activity is not physiologically associated with green color and the color characteristics can be lost without modifying lipolysis. Whether or not the increased lipolytic activity of mutants 1-10 and 2-10 is the result of an ultraviolet induced change has yet to be deter-



FIG. 2. *P. roqueforti* strain 2 grown on milk with and without added iron.

Flask 1	0.0 mg. FeCl <sub>3</sub> added
" 2	0.5 " " "
" 3	1.0 " " "
" 4	2.0 " " "
" 5	4.0 " " "

mined. Table 2 shows that proteolysis, like lipolysis, is not physiologically associated with green coloration.

During an investigation of the metals associated with the green coloration of normal *P. roqueforti*, it became evident that the iron requirements of the normal molds and the white mutants were quite different. Figure 2 shows the effect of iron (FeCl<sub>3</sub>) added to whole milk on sporulation of strain 2. Table 3 gives the

TABLE 3

*The influence of iron on the weight of mycelium produced by P. roqueforti 2 and 2-10 on whole milk at 25° C*

FeCl <sub>3</sub> added	Dry weight of mycelium	
	Strain green (2) <sup>a</sup>	white (2-10)
(mg./100 ml.)		
none	0.74	1.01
0.5	0.76	1.11
1.0	0.85	0.95
2.0	1.04	1.02
4.0	1.13	1.07

<sup>a</sup> Mycelium from flasks shown in fig. 2.

dry weight of the mycelium of strain 2 in each of the flasks in figure 2, as well as the weight of strain 2-10 that was grown at the same time. Similar results were obtained with strains 1 and 1-10. From these data it is evident that the white mutants will grow well in milk without additional iron, whereas the green parents grow and sporulate poorly unless iron is added. Finely divided metallic iron and the iron in ferric citrate, ferric lactate, ferric chloride and ferric sulfate was available.

#### DISCUSSION

The results of these experiments indicate that the green color of *P. roqueforti* can be lost permanently by induced mutation without markedly changing the lipolytic and proteolytic activity or the growth of the mold. Hence, roquefort-type cheese made with the white molds would be lacking the green venation usually associated with such cheese, but probably would be normal in other respects. Mold-ripened cheese made from the white mutants would be desirable for the manufacture of cheese spreads and blends and should appeal to consumers who are prejudiced against eating the conventional roquefort-type cheese which to them appears "moldy." That at least one of the white mutants can be used for the manufacture of roquefort-type cheese has been shown by Jezeski *et al.* (2).

It should be possible to produce mold-ripened cheese with new flavors and textures by use of *P. roqueforti* or other molds, the lipolytic or proteolytic activity of which has been modified by mutation. It could be that the increased lipolytic activity of mutant 2-10 (table 1) is due to such a mutation, or that a more lipolytic strain has been selected by isolation of single spores from the parent culture.

The high iron requirements for good growth and sporulation of *P. roqueforti* might be very significant in the manufacture of mold-ripened cheese. The fact that milk might not contain enough iron for good growth of the green molds was noted first on pasteurized milk obtained from the University Dairy. However, it was found that not all samples of milk from the Dairy were deficient in iron; apparently, contamination from utensils provided enough of the essential metal in some instances. All samples of milk that were obtained directly from the cow and never were in metal utensils were iron-deficient for the green molds but not for the white mutants. It is probable that lack of available iron in the milk sometimes may result in poor growth of the mold in roquefort-type cheese. Because of the low iron requirements of the white mold, milk probably contains enough iron for the manufacture of "white mold" cheese. It is interesting that the absence of color in the spores of the mutants should decrease the amount of iron needed for growth and that the green mold at low levels of iron both grew and sporulated poorly. The relationship between pigmentation of the spores and growth of the mold is being studied.

There is evidence that the color in *P. roqueforti* is determined by a single gene since only white heterocaryons have been obtained from various combinations of ten colorless mutants. If color were determined by more than one gene it would be unlikely that the same gene would have been hit in each of the ten

mutants. That heterocaryosis does occur has been proved by producing stable pale green heterocaryons as a result of anastomosis between a colorless mutant and a normal green parent. Anastomosis of hyphae has been observed frequently by microscopic observation. Nevertheless, since all of the factors involved in the synthesis of heterocaryons are not known, definite conclusions in regard to the genetics of the green color of *P. roqueforti* spores should not be made at this time.

The practical applicability of these findings is being developed through the Wisconsin Alumni Research Foundation in cooperation with the Division of Dairy Husbandry, University of Minnesota.

#### SUMMARY AND CONCLUSIONS

Strains of *Penicillium roqueforti* with white spores have been obtained by ultraviolet-induced mutation of the green mold.

The white mutants were produced without marked changes in the lipolytic, proteolytic or growth rates of the mold.

Evidence is presented to show that normal *Penicillium roqueforti* probably requires more iron for good growth than may be present in mixed samples of milk. Milk that was produced without contact with a metal container was a poor medium for the green mold unless iron was added.

The white mutants required less iron for growth than the green parents and grew normally in milk to which no iron had been added.

Apparently one gene is responsible for the green pigmentation of the spores of *Penicillium roqueforti*.

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# COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Atlantic City, N. J.—Oct. 16, 1950

Teams from 26 State Agricultural Colleges participated in this, the sixteenth annual contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

## ALL PRODUCTS

### Individuals

- \*1. Edward Schuch, Iowa State College
- \*2. Thomas E. Gilmore, Mississippi State College
3. Willis E. Parkin, University of Connecticut
4. Duane Osam, Iowa State College
5. James Stanton, Ohio State University
6. William E. Sandine, Iowa State College
7. Graham E. Hall, University of Connecticut
8. James C. Sutherland, Michigan State College
9. Richard H. Andrews, Mississippi State College
10. Jack Davis, Mississippi State College

### Teams

1. Iowa State College
2. Mississippi State College
3. University of Connecticut
4. Michigan State College
5. Ohio State University
6. University of Georgia
7. Cornell University
8. Oklahoma A. & M.
9. Purdue University
10. North Carolina State College

\* Tied for first place; tie broken in favor of Edward Schuch on flavor score of 60.67 compared with flavor score of 67.84 for Mr. Gilmore.

## BUTTER

### Individuals

1. Farris E. Ashe, University of Tennessee	11.25
2. Richard H. Andrews, Mississippi State College	11.67
3. Edward Schuch, Iowa State College	12.50
4. Kenneth Van Patten, Michigan State College	13.17
5. Richard J. Stucky, University of Minnesota	13.67
6. Edward B. Hanna, West Virginia University	13.67
7. Thomas E. Gilmore, Mississippi State College	14.17
8. William E. Sandine, Iowa State College	14.34
9. Donald G. Sickafoose, Ohio State University	14.67
10. George Farrell, Purdue University	15.17

### Teams

1. Iowa State College	42.18
2. Mississippi State College	44.01

3. Purdue University . . . . .	46.92
4. University of Minnesota . . . . .	48.17
5. University of Tennessee . . . . .	50.42
6. Ohio State University . . . . .	50.67
7. University of Maryland . . . . .	53.34
8. Michigan State College . . . . .	53.35
9. Cornell University . . . . .	56.35
10. University of Connecticut . . . . .	57.51

## CHEESE

*Individuals*

1. Thomas E. Gilmore, Mississippi State College . . . . .	23.55
2. Edward Schuch, Iowa State College . . . . .	24.00
3. Kenneth Van Patten, Michigan State College . . . . .	25.58
4. Duane Osam, Iowa State College . . . . .	25.58
5. James C. Sutherland, Michigan State College . . . . .	26.33
6. James Stanton, Ohio State University . . . . .	26.67
7. Earl M. Harvey, University of Nebraska . . . . .	26.76
8. Ervin C. Hamme, Pennsylvania State College . . . . .	27.01
9. Willis E. Parkin, University of Connecticut . . . . .	28.14
10. Graham E. Hall, University of Connecticut . . . . .	28.33

*Teams*

1. Iowa State College . . . . .	78.92
2. Michigan State College . . . . .	85.49
3. Purdue University . . . . .	88.52
4. University of Connecticut . . . . .	91.64
5. Mississippi State College . . . . .	94.33
6. Ohio State University . . . . .	95.18
7. North Carolina State College . . . . .	100.05
8. Cornell University . . . . .	101.68
9. University of Georgia . . . . .	102.58
10. University of Nebraska . . . . .	103.68

## ICE CREAM

*Individuals*

1. Richard H. Andrews, Mississippi State College . . . . .	25.00
2. Jack Davis, Mississippi State College . . . . .	26.00
3. Hilmer H. Schuelke, Texas A. & M. . . . .	28.33
4. Joe Otto Brown, North Carolina State College . . . . .	29.00
5. Stanley L. Ruxton, University of Connecticut . . . . .	31.00
5. Willis E. Parkin, University of Connecticut . . . . .	31.00
7. Robert W. Skinner, University of Tennessee . . . . .	31.25
8. Farris E. Ashe, University of Tennessee . . . . .	31.50
9. Graham E. Hall, University of Connecticut . . . . .	32.00
9. James A. Paul, Cornell University . . . . .	32.00
9. James Stanton, Ohio State University . . . . .	32.00
9. Aaron B. Karas, Cornell University . . . . .	32.00

*Teams*

1. Mississippi State College . . . . .	86.87
2. University of Connecticut . . . . .	94.00



3. Texas A. & M. . . . .	98.58
4. University of Tennessee . . . . .	98.75
5. Cornell University . . . . .	104.00
6. University of Georgia . . . . .	104.17
7. Iowa State College . . . . .	104.50
8. Michigan State College . . . . .	104.67
9. North Carolina State College . . . . .	107.50
10. Ohio State University . . . . .	108.50

## MILK

*Individuals*

1. Willis E. Parkin, University of Connecticut . . . . .	15.32
2. Gale G. Ripma, Michigan State College . . . . .	18.72
3. Edward Schuch, Iowa State College . . . . .	23.42
4. James C. Otto, University of Minnesota . . . . .	23.84
5. Thomas E. Gilmore, Mississippi State College . . . . .	24.60
6. H. Douglas Cope, Ohio State University . . . . .	24.75
7. Harold Windlam, University of Georgia . . . . .	25.06
8. Jack E. Conrad, University of Maryland . . . . .	26.00
9. James Stanton, Ohio State University . . . . .	26.05
10. Roger B. Thompson, University of Massachusetts . . . . .	27.45

*Teams*

1. University of Connecticut . . . . .	73.17
2. Michigan State College . . . . .	81.40
3. Iowa State College . . . . .	81.91
4. University of Georgia . . . . .	85.97
5. Ohio State University . . . . .	87.40
6. Mississippi State College . . . . .	87.54
7. Oklahoma A. & M. . . . .	88.66
8. University of Maryland . . . . .	91.20
9. Texas Technological . . . . .	93.72
10. University of Nebraska . . . . .	95.89

# NATIONAL INTERCOLLEGIATE DAIRY CATTLE JUDGING CONTEST NATIONAL DAIRY CATTLE CONGRESS—1950

## Waterloo, Iowa

### TEAM RANK—ALL BREEDS

1. Ohio	2081
2. Iowa	2026
3. Pennsylvania	2022
4. Kentucky	2000
5. Calif. State Polytechnic	1980
6. Maryland	1978
7. Missouri	1966
8. Ontario Agr. College	1947
9. Cornell	1924
10. Texas Tech.	1922

### HIGH INDIVIDUALS IN JUDGING ALL BREEDS

1. Herman Rickard, Ohio State	702
2. Carl Young, Ohio State	700
3. G. J. Lyon, Iowa State	695
4. James Fish, Pennsylvania State	690
5. W. Earle Roger, Ontario Agr. College	689
6. Cecil Burnette, Kentucky	685
7. James Moxley, Maryland	683
8. Ben Broesma, Calif. Polytechnic	681
9. T. A. Burgeson, Missouri	680
10. William E. Davis, Jr., Ohio State	679

### AYRSHIRE

<i>Teams</i>		<i>Individuals</i>	
1. Calif. Polytechnic	425	1. Lawrence Barba, Calif. Poly.	146
2. N. Carolina State	423	2. (W. Earle Roger, Ontario Agr.	144
3. Kentucky	415	3. (Engimar Sveinsson, Wash. State	144
4. Pennsylvania	405	4. (Max Sink, N. Carolina	143
5. Maryland	402	5. (Robert Johnson, Calif. Poly.	143
6. Ohio State	398	6. (James Martin, Kentucky	143
7. Univ. of Missouri	396	7. (Tommie McPherson, N. Carolina	142
8. Washington State	394	8. (Richard Riggs, Purdue	142
9. Ontario Agr. College	392	9. (M. D. Rinner, Iowa State	142
10. (Purdue	391	10. Arthur Korte, Missouri	141
11. (Kansas	391		

### BROWN SWISS

1. Iowa State	431	1. Herman Rickard, Ohio State	148
2. Ohio State	426	2. (Engimar Sveinsson, Wash. State	147
3. Univ. of Ill.	404	3. (William Shenton, Iowa State	147
4. Ontario Agr. College	391	4. (M. D. Rinner, Iowa State	146
5. Washington State	390	5. (Ben Broesma, Calif. Poly.	146
6. Kentucky	389	6. (Carl Young, Ohio State	146
7. Univ. of Wis.	388	7. Cecil Burnette, Kentucky	142

8. (Maryland . . . . .)	387	8. (James Fish, Penn. State . . . . .)	141
9. (Calif. Poly. . . . .)	387	9. (Ray Briggs, Cornell . . . . .)	141
10. Connecticut . . . . .	380	10. Earl Spurrier, Maryland . . . . .	140

## GUERNSEY

1. Pennsylvania State . . . . .	433	1. (Herman Rickard, Ohio State . . . . .)	148
2. Ohio State . . . . .	431	2. (James Fish, Penn. State . . . . .)	148
3. Iowa State . . . . .	422	3. (M. D. Rinner, Iowa State . . . . .)	148
4. Ont. Agr. College . . . . .	419	4. (Carl Young, Ohio State . . . . .)	147
5. Texas Tech. . . . .	418	5. (James Moxley, Maryland . . . . .)	147
6. Kentucky . . . . .	417	6. Charles Harding, Penn. State . . . . .	146
7. Purdue . . . . .	413	7. Vestal Shipman, Texas Tech. . . . .	145
8. Cornell . . . . .	410	8. (W. Earle Roger, Ont. Agr. College . . . . .)	144
9. Maryland . . . . .	404	9. (T. A. Burgeson, Missouri . . . . .)	144
10. Okla. A & M . . . . .	398	10. Robert Peterson, Purdue . . . . .	143

## JERSEY

1. Ohio State . . . . .	418	1. E. B. Morgan, Louisiana State . . . . .	147
2. (Texas Tech. . . . .)	417	2. Robert Strickler, Kansas . . . . .	144
3. (Cornell . . . . .)	417	3. (Tommie Hewlett, Texas Tech. . . . .)	143
4. Calif. Poly. . . . .	416	4. (Don House, Cornell . . . . .)	143
5. (Penn. State . . . . .)	405	5. (G. J. Lyon, Iowa State . . . . .)	143
6. (Louisiana State Univ. . . . .)	405	6. (Charles Harding, Penn. State . . . . .)	142
7. Missouri . . . . .	401	7. (Ben Broesma, Calif. Poly. . . . .)	142
8. Iowa State . . . . .	399	8. (C. B. Smith, Texas A & M . . . . .)	142
9. Okla. A & M . . . . .	394	9. (T. A. Burgeson, Missouri . . . . .)	142
10. Kansas . . . . .	393	10. (W. Earle Roger, Ont. Agr. College . . . . .)	141
		11. (W. E. Davis, Jr., Ohio State . . . . .)	141
		12. (Herman Rickard, Ohio State . . . . .)	141

## HOLSTEIN

1. Maryland . . . . .	415	1. William Shenton, Iowa State . . . . .	146
2. Kentucky . . . . .	413	2. James Fish, Penn. State . . . . .	144
3. Ohio State . . . . .	408	3. (William Curry, Maryland . . . . .)	142
4. Penn. State . . . . .	407	4. (Ward Richter, Wisconsin . . . . .)	142
5. Iowa State . . . . .	402	5. (G. J. Lyon, Iowa State . . . . .)	141
6. Missouri . . . . .	399	6. (Don House, Cornell . . . . .)	141
7. Illinois . . . . .	397	7. (W. E. Davis, Jr., Ohio State . . . . .)	140
8. Texas A & M . . . . .	394	8. (Carl Young, Ohio State . . . . .)	140
9. Univ. of Wis. . . . .	388	9. (Ed Thomason, Okla. A & M . . . . .)	139
10. Cornell . . . . .	385	10. (Cecil Burnette, Kentucky . . . . .)	139
		11. (T. A. Burgeson, Missouri . . . . .)	139
		12. (Robert Hertzog, Missouri . . . . .)	139

## NEW MEMBERS FOR 1950

### ARKANSAS

HORTON, OTIS H., Dept. of Animal Industry,  
Univ. of Arkansas, Fayetteville

### CALIFORNIA

BOYLE, KENNETH D., Calif. State Polytechnic  
College, San Luis Obispo  
FREY, LELAND S., Federal Bldg., Red Bluff  
PELISSIER, C. L., Univ. of California, Davis  
WILCOX, DONALD F., c/o Golden State Co.,  
Newman  
YOUNG, JAMESON, 933 Stockton St., San Fran-  
cisco 8

### COLORADO

CALVERT, PHILIP B., c/o Robinson Dairy Inc.,  
2550 Larimer St., Denver  
HILL, HAROLD J., Veterinary Hospital, Colo.  
A & M College, Ft. Collins

### CONNECTICUT

AVAMPATO, JAMES E., Box U-40, Univ. of Con-  
necticut, Storrs  
BUSCHNER, FREDERICK A., Star Route, Mans-  
field Center  
EASTERBROOKS, H. L., Box U-39, Univ. of  
Conn., Storrs  
HELLER, PAUL, Woodbridge  
RUCH, ERWIN, Inredeco, Inc., 1 Atlantic St.,  
Stamford

### FLORIDA

ANTEL, RICHARD K., 621 N.E. 30th Terrace,  
Miami  
HAMMER, B. W., 369 W. Arlington Ave.,  
Sarasota  
LEWIS, JOHN, 900 N.W. 130th St., Miami  
SAYLOR, NATHANIEL, Box 67, Florida A & M  
College, Tallahassee  
VAN LANINGHAM, GEORGE L., 906 Odessa Dr.,  
Jacksonville

### GEORGIA

MANLEY, NEIL, Route 3, Athens  
SELL, OTTO E., Georgia Expt. Station, Experi-  
ment  
WILBANKS, W. H., Winder

### IDAHO

SENFTEN, EUGENE R., 2015 N. 19th St., Boise

### ILLINOIS

BIANCO, LOUIS S., 2146 Illinois Rd., North-  
brook  
CASH, J. G., 120 Davenport Hall, Univ. of Il-  
linois, Urbana  
GUTHRIE, RICHARD S., 812 N. 4th St., De Kalb  
MARSHALL, M. J., Feed Dept., Quaker Oats,  
Chicago 54  
NELSON, WILLARD O., 452 Davenport Hall, Uni-  
versity of Ill., Urbana  
ORMOND, N. H., 15 N. Grant, Sullivan  
SISLER, WILLIAM, Ohio

### INDIANA

EAKLE, DON E., Farmers' Cooperative Cry.,  
211-15 No. Washington St., Crawfordsville

### IOWA

JONES, GAIL M., 411 West 4th St., Storm Lake  
TAMSMA, A. F., Dept. of Dairy Industry, Iowa  
State College, Ames  
VITTENGLE, JOHN G., 117 Rapids Ave., S.W.,  
Cedar Rapids

### KANSAS

BONEWITZ, RALPH, 1005 Ratone, Manhattan  
KNOX, JAMES E., Mound Valley Branch-Ex-  
periment Sta., Mound Valley

### KENTUCKY

OLDS, DURWARD, Dairy Dept., Univ. of Ky.,  
Lexington

### LOUISIANA

ALFORD, DR. JOHN A., Dept. of Bacteriology,  
Animal Ind. Bldg., L.S.U., Baton Rouge

### MARYLAND

BOOK, JAMES H., U.S.D.A., Bureau of Dairy  
Ind., Beltsville  
HARTMAN, ARTHUR M., U.S.D.A., Bureau of  
Dairy Industry, Beltsville  
NISONGER, JOSEPH W., 3334 Lancer Dr.,  
Hyattsville  
RACZ, STEPHEN J., Goldsboro

### MASSACHUSETTS

GROSSMITH, FREDERICK J., JR., R.F.D. 376,  
Foxboro  
PARSONS, CLARENCE H., 37 Farview Way, Am-  
herst  
PUTNEY, CHESTER C., 275 Cherry St., Shrews-  
bury

### MICHIGAN

BOVING, STIG G., The Borden Co., 3600 E.  
Forest, Detroit 7  
PETERSON, MERRITT, McDonald Cooperative  
Dairy Co., Dearborn  
ROBINSON, ROBERT F., 8162 Burnette, Detroit  
4  
SORENSEN, College Station, Berrien Springs  
VAN HOOVEN, JACOB, Jenison

### MINNESOTA

RUDNICK, ARTHUR W., JR., 1464 Holton, St.  
Paul 4

### MISSOURI

ANGEVINE, N. C., 310 Russell Blvd., c/o Meyer-  
Blanke Co., St. Louis 4  
BURNS, LOREN V., 1025 Dwight Bldg., 1004  
Baltimore Ave., Kansas City 6  
CAINE, RICHARD, Producers' Creamery Co.,  
Monett

## NEVADA

ADAMS, CHARLES C., JR., c/o Animal Husbandry Dept., Univ. of Nevada, Reno

## NEW HAMPSHIRE

STEWART, ROBERT D., American Guernsey Cattle Club, Peterborough

## NEW JERSEY

COSTELLO, THOMAS JOSEPH, 223 Water St., Perth Amboy

## NEW YORK

CUNNINGHAM, L. F., Warren Hall, Cornell Univ., Ithaca

FRAM, HARVEY, National Dairy Research Labs., Oakdale, L. I.

GIBBONS, AUSTIN P., 27 Elm St., Delhi

HINKLEY, VINCENT G., R.D. 4, Slaterville Rd., Ithaca

HOWARD, HARTLEY A., 350 Madison Ave., New York 17

JENKS, STANTON F., Ravena

LUTHER, HERBERT G., c/o Cha. Pfizer Co., 11 Bartlett St., Brooklyn 6

McMILLAN, WILLIAM D., Terrace Hill, c/o Coop. G.L.F. Exchange, Inc., Ithaca

MURPHY, JAMES M., N. Y. State Veterinary College, Ithaca

ROSA, HAROLD, R.D. 4, Ithaca

SCHAIN, PHILIP, D.Sc., Halloran V. A. Hospital, Staten Island 1

SWANNER, ROY O., N. Y. State Dept. of Health, 412 Rogers Bldg., Glens Falls

PENCE, JOHN T., Box 236, Massena

PTIZ, EDWARD W., 600 N. Franklin St., Syracuse

LOOMIS, VADER M., Mannsville

VAN PELT, M. DEAN, Box 128, Kinderhook

## NORTH CAROLINA

AURAND, L. W., Dept. of Animal Industry, State College Sta., Raleigh

JARVIS, RAY N., Mtn. Expt. Station, Waynesville

## OHIO

COMSTOCK, C. R., Box 1836, Columbus

HARPER, W. J., Dept. of Dairy Technology, The Ohio State Univ., Columbus

HOOVER, A. S., 461 E. Sandusky St., Findlay

HENKE, J. RICHARD, c/o Sidney Dairy Products, Inc., 507 N. Miami Ave., Sidney

KEENEY, DAVID G., M & R Dietetic Labs., Inc., 585 Cleveland Ave., Columbus

REINKE, EUGENE F., 1315 Delta Ave., Cincinnati 8

ROBERTS, JAMES O., 386 S. Terrace Ave., Columbus 4

RYSER, FRED C., Ohio Swiss Cheese Assn., Inc., Sugarcreek

## OREGON

COVINGTON, JUNIUS L., Dept. of Dairy Husbandry, Oregon State College, Corvallis

HAGO, OSCAR, 401 Education Center Bldg., 220 W. Alden St., Portland

JOHNSON, GERALD, 3235 Portland Rd., Salem

## PENNSYLVANIA

CONNOLLY, DR. JOHN J., 307 Philadelphia St., Indiana

FOWLER, CLYDE A., 3227 Gaylord Ave., Pittsburgh 16

HALL, CLYDE N., Room 213, Dairy Bldg., State College

HENNING, WILLIAM LEWIS, 203 Agr. Bldg., State College

WATTON, ALFRED M., February Hill Farm R. D. Chalfont, Bucks City

WILLIS, LEWIS O., 316 N. 9th St., Reading

## RHODE ISLAND

BUCHANAN, JOHN O., R. I. State College, Kingston

## SOUTH CAROLINA

DAVENPORT, LT. FORREST N., Section 3, A.S.U. 3431, U. S. Army Hospital, Ft. Jackson

## TENNESSEE

HATLER, FRED J., West Tenn. Artificial Breeding Assoc., Yorkville

MUNDT, J. ORVIN, Bacteriology Dept., Univ. of Tenn., Knoxville

## UTAH

CLAYBURN, L. DEE, B 54 Victory Rd., Washington Terrace, Ogden

## VERMONT

ATHERTON, HENRY V., 14 University Terrace, Burlington

REED, RALPH E., 19 Maple St., Essex Junction

## VIRGINIA

BROOKS, JAMES P., 305 King Ave., Waynesboro

EDWARDS, MARION, Univ. of Delaware, Ashland

HOBSON, CURTIS, Box 6, Salem

PAIS, A. A., State Health Dept., Richmond

## WASHINGTON

LOGEN, THOMAS, Rte. 2, Box 12, East Stanwood

SMITH, RICHARD A., 1417 N. Grand, Pullman

## WEST VIRGINIA

HARE, JOHN H., Dept. of Agr. Biochemistry, West Virginia Univ., Morgantown

## WISCONSIN

CASIDA, L. E., Dept. of Genetics, Univ. of Wis., Madison

KALK, FAY E., 1232 2nd Ave., Antigo

McINTIRE, J. M., 500 So. Story St., Appleton

MIERSCH, RAYMOND E., 460 N. Sidney St., Kimberly

RASMUSSEN, HAROLD L., 2850 Barlow St., Madison 5

WALLACE, ALEXANDER E., 638 S. Lake St., Neenah

WIDDIFFIELD, W. F., 211 W. 3rd St., Shawano

## FOREIGN MEMBERS

BURGUERA, D., GERMAN G., Bella Vista No.  
161-MAC, Maracaibo, Venezuela, S. A.  
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Services, C. P. and Berar, Nagpur, India  
LARA, EDUARDO, Avenida Angelica 1803, Sao

Paulo, S. P. Brazil, S. A.  
NIELSEN, SIGURD, Johan Wilmannsvej 34,  
Lyngby, Denmark  
SHAWYER, S. R., The Borden Co., Ltd., To-  
ronto, Ont., Canada  
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fabried Friesland Leeuwarden, Netherlands

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# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

F. E. NELSON, Editor  
Ames, Iowa

## MILK AND MILK PRODUCTS

### MILK PRODUCTION

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B. F. CASTLE, Executive Director  
1001 Fifteenth Street, N.W., Washington 5, D. C.

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*Sanitation and Cleansing*



# ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

## BOOK REVIEW

1. **Judging Dairy Products**, 2nd ed. J. A. NELSON AND G. M. TROUT. Olsen Publishing Co., Milwaukee, Wis. 494 pp. 1948.

This edition not only includes the judging of more dairy products than the original book, but the information is much more detailed. The score cards for milk, ice cream, butter and cheese have been standardized to a flavor score of 45 points. The flavor scoring guides given for each product are very helpful in instructional work and are improved over those given in the old book.

The judging of chocolate milk, skimmilk, goat's milk, fermented milk, evaporated milk, condensed milk, dry milk solids and cream are handled very well, also.

Different types of cheese with respect to process of manufacture, characteristics and judging or grading are discussed. The Cheddar or swiss, brick, limburger, blue-veined, cottage and cream cheeses are included.

Ice cream also is considered in more detail, with special flavors and sherbets receiving attention.

This is an excellent manual for the plant operator as well as the judge of dairy products, as the cause of defects in dairy products and the remedies given should find practical application in most dairy plants. W. S. Rosenberger

## ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

2. **Brucellosis therapy: studies on the effect of streptomycin and sulfadiazine in experimental brucellosis in guinea pigs.** L. S. HOLM AND S. H. McNUTT, Univ. of Wis., Madison. Am. J. Vet. Research, 10, 37: 336-340. Oct., 1949.

Male guinea pigs inoculated with 1 million *Brucella* organisms were used as the test animals. Para-amino-benzoic acid did not alter the course of the infection. Streptomycin or sulfadiazine had a slight effect when given singly and a definite curative effect when given together. When treatments were initiated on the day of infection

practically 100% bacteriologic cures developed. Treatment started at 2, 5 or 10 d. after infection was also highly effective. Doses of 10,000 units streptomycin plus 120 mg. sulfadiazine given once daily were just as effective as 5,000 units streptomycin plus 120 mg. sulfadiazine divided into 2 or 5 doses/d. No toxicity was observed as a result of the treatments. E. W. Swanson

3. **Epizootiology of mastitis: the relative importance of extended exposure and of age in the spread of *Streptococcus agalactiae* infection.** R. ORMSBEE AND O. W. SCHALM, Univ. of Cal. Berkeley. Am. J. Vet. Research, 10, 37: 306-313. Oct., 1949.

Data from a large commercial dairy herd collected over more than 3 yr. time were used to check the validity of the "age factor" hypothesis of mastitis susceptibility. Two outbreaks of *S. agalactiae* infection occurred after the herd had been freed of such infection, which exposed a total of 629 clean cows of various ages over a period of 4 mo. The incidence of infection from 1st to 5th or higher lactation was respectively 9.5, 19.0, 21.1, 20.3 and 23.7%. Under extended exposure the same herd prior to eliminating the infection had had age incidence of 16.7, 23.7, 44.8, 71.5 and 76.6% for 1st to 5th or higher lactation. When the clean cows were exposed, 205 had been infected previously and cured by chemotherapy and 236 in a comparable group had not been infected previously. The resulting incidence of infection in these groups was 21.95 and 19.92%, respectively, while according to the "age factor" hypothesis nearly all of the cows in the former group should have been reinfected. These data indicate that degree of exposure is of primary importance in establishing *S. agalactiae* infections and that clean cows of all ages or previous mastitis history react similarly to the same exposure. E. W. Swanson

4. **Over de behandeling van streptococcenmastitis met penicilline en de bestrijding van streptococcenmastitis met penicilline en autovaccin.** (The penicillin treatment, a reliable therapy against streptococcal mastitis.) (English summary.) O. BOSGRA, R. POST AND D. REMPT.



Netherlands Milk & Dairy J., 3, 3: 155-161. July-Sept., 1949.

Experiments performed with penicillin and vaccine treatments are described. Five seriously infected herds of cattle with a total of 100 cows were investigated, in which 20% of the quarters of the udder were found positive. These quarters were treated by intramammary injection with 25,000 to 50,000 units of penicillin, depending on the amount of milk produced. This dosage was given in 2 lots with a 24-hr. interval, using distilled water as a solvent. Investigation of the quarters 1 wk. later showed that the mastitis-positive percentage had dropped to 4%. Cattle free from mastitis were treated at the same time with a mixed vaccine prepared from the mastitis strains involved in the experiment. Six mo. later, 13% of the penicillin-treated cows were found positive, giving a 9% increase. The vaccinated cows now had a mastitis percentage of 9%, also a 9% increase.

The following conclusions were drawn: penicillin treatment is a reliable therapy against streptococcal mastitis. No noticeable immunity is produced, either in animals which have been cured of infection or in those inoculated with a specific vaccine. Cattle, suffering at a given time from a streptococcal infection of the udder, are not more sensitive to an infection by the streptococcus concerned than cattle which are at that time free from this infection. A. F. Tamsma

5. *Het onderzoek op tuberculose bij het rundvee in Nederland en enkele beschouwingen over de huidige stand ervan.* (Testing cattle for bovine tuberculosis in the Netherlands and some views of the present situation.) (English summary.) C. F. VAN OIJEN AND G. B. R. WILLEMS, State University of Utrecht, Holland. Netherlands Milk & Dairy J., 3, 2: 91-112. April-June, 1949.

After a short historical review of the campaign against bovine tuberculosis in the Netherlands, the present situation is described. In the past, farmers volunteered in local organizations, which later were united into Provincial Unions. A few months after the end of this war the eradication of tuberculosis in cattle was regulated by law. The state and local authorities combine with farmers' organizations and organizations of the dairy industry to meet the costs and losses for individual farmers. In many districts milk from tuberculosis-free herds warrants a premium.

The measures consist of the following: (1) a preliminary clinical examination and elimination from the herd of obviously sick animals, (2) intradermal (or conjunctival) tuberculation of the whole stock, (3) segregation of reactors from

non-reactors, marking of reactors, (4) clinical examination and microscopical investigation of secreta, to detect open cases of tuberculosis (in special cases the microscopical examination is confirmed by the culture of tubercle bacilli and/or inoculation of guinea pigs), (5) immediate slaughtering of all open cases, (6) raising of calves so called tuberculosis-free, i.e., feeding with milk from cows known to be free from tuberculosis, protection against any possibility of infection, (7) compulsory pasteurization of skim milk and whey destined for feeding of live stock, (8) re-examination of non-reactors at least every year; there is a growing tendency to repeat the tuberculation and the clinical examination twice a year and (9) progressive elimination of reactors and their replacement by tuberculosis-free animals.

During the last years of the war and the first years thereafter, unfavorable conditions caused some positive reactors in some herds which were thought to be free from tuberculosis. At present, a very large number of herds, especially in breeding centers, are free from tuberculosis. The authors expect that tuberculosis in cattle will be reduced to a minimum in a few years.

A. F. Tamsma

6. *The response of cattle to penicillin preparations following intramuscular injection.* E. V. MORSE, Cornell Univ., Ithaca, N. Y. Am. J. Vet. Research, 10, 37: 314-317. Oct., 1949.

The effectiveness of various penicillin preparations was measured by assaying blood plasma and urine at intervals following intramuscular injection of 1 and 1.5 million units. Crystalline penicillin G in aqueous solution was absorbed and excreted rapidly, only traces being found in plasma at 7 hr. Penicillin in oil and beeswax maintained more than trace levels of penicillin in plasma for 48 hr. Procaine penicillin G in oil gave practically the same results. Procaine penicillin in peanut oil and 2% aluminum monostearate maintained good blood and urine concentration for 72 hr. and traces were found up to 120 hr. Single injection sites were more efficacious than double sites. E. W. Swanson

7. *The relative importance of antibodies and Vitamin A in preventing disease in young calves.* (Abs.) F. BLAKEMORE *et al.*, Inst. of Animal Path. and Dunn Nutritional Lab., Cambridge. Biochem. J., 42, 2: xxx. 1948.

When colostrum, known to be rich in vitamin A as well as containing antibodies, is not given to young calves, "white scour" often results. Attempts to prevent this abnormality by giving vitamin A were not successful, but when calves were

inoculated with precolostrum, demonstrated to contain antibodies in concentrated form, they were protected. The protective value of colostrum is assumed to be associated with its globulin content.

A. O. Call

Also see abs. no. 50.

## BUTTER

O. F. HUNZIKER, SECTION EDITOR

**8. Konsistenz der Butter. Teil II. (The body characteristics of butter. Part II.)** English summary. W. MOHR AND F. SCHULZ. *Die Milchwissenschaft*, 3, 12: 362-366. Dec., 1948.

Butter cutting trials with Alfa butter at temperatures of from 15 to 22° C. indicated that the resistance to cutting varied inversely to the temperature of the butter; the cutting velocity being constant at 0.1 cm./cc. By increasing the cutting velocity from 0.0001 to 0.25 cm./sec. a measurable increase in the force required to cut the butter was observed. The slope of curves obtained was similar in the case of normal acid of "layer type" Alfa winter butter. The slope for crumbly, ripened cream Alfa winter butter was slightly more steep than for normal Alfa winter butter. Best results were obtained when the butter samples were cut at 10° C. at a velocity of 0.01 cm./sec. At greater velocities the response of the balance was too slow, whereas at lower velocities the time required was rather long and the butter warmed up to room temperature.

The body of normal and of crumbly butter, tempered to 10° C., was compared by determining the "break point" of a stick of butter 10 × 2 × 2 cm. with the force applied in the center of the stick at a velocity of 0.1 cm./sec. Crumbly butter had a much lower "break point", i.e., it required less force to break the stick, than did normal butter. Normal butter containing air pockets gave values similar to those of crumbly butter. The defect "layer type" Alfa butter could not be detected by the above method.

The "break point" method can be used to advantage with crumbly winter butter only.

Measurements of the firmness of butter by means of comparison of the cutting resistance with the cone flow point and with the "break point" can serve as a guide for detecting crumbliness in butter. For greasy, soft summer butter comparisons of cutting resistance to cone flow point are of value.

I. Peters

**9. Aktuelle kvalitetsproblemer ved produksjon av smør. (Quality problems in the production of butter.)** *Meieriposten*, 38, 27: 473-475. July, 1949.

In the annual report from Norske Meieriers

Salgscentral for 1948, Olav Benterud summarizes the work done on quality problems. Results from 4610 churnings at 37 creameries were studied for 2.5 yr. The fresh butter was judged at 2.5 to 3, 5 to 6, 7.5 to 8 and 9 to 10 mo. The temperature at judging was 13 to 14° C. Six judges officiated. A comparison was made between the quality of butter from low and high acid cream. The high acidity was preferable when the pH was regulated. For fresh butter, a pH of 5 to 6 gave the best results and at this pH the average score was 10.7 for non-coagulated cream and 11.1 for coagulated cream. With a pH over 6, the scores were 10.7 and 11.0. The lowest average score was that for sweet cream butter, namely 10.4.

With storing under refrigeration, the butter from neutralized cream was just as high in quality as butter from low acid cream. Sweet cream butter, at 10 mo. storage, had decreased only 0.3 point, indicating very good keeping quality. In butter from non-coagulated cream and a pH over 6 the decrease in score was 0.6 point, and at a pH of 5 to 6, the decrease was 1.3 points. For coagulated cream the decrease was 0.8 point with pH over 6, 1.8 with a pH content of 5 to 6 and 2.4 points with the pH content under 5. This was for butter held in cold storage for 10 mo.

There was a greater decrease in score for ripened, coagulated, cream butter, than in unripened, non-coagulated, cream butter but since the score of the fresh butter made from ripened coagulated cream was larger than that from uncoagulated cream, the final scores were approximately the same when the pH was over 6 and between 5 and 6.

The results for butter from high acid cream with a pH under 5 would indicate that this butter is unsuitable for storage purposes. Earlier experiments have shown a direct relation between the pH value in butter and its keeping quality. The most suitable pH seems to be between 5.5 and 6, where butter is to be stored for 6 mo. The best butter had a pH from 5.51 to 6.21.

The butter was scored immediately after taking it out of storage and again after it had been stored at 13 to 14° C. for 2 wk. There usually was a decrease in score between that of the butter scored immediately after removing it from storage and that of butter scored after it had been left out of storage for 2 wk. There seemed to be no greater decrease in score during this 2-wk. period of the 10-mo. storage butter than in the fresh butter. If an oxidized defect is not present when the butter is removed from storage, it is not likely to develop before the butter is consumed.

There seemed to be no difference in the quality of washed and unwashed butter. Experiments were made using sodium bicarbonate or hydrate.

This was used without mixing it with the butter salt.

Comparisons were made by 78 churnings being done with a roller-type churn and 81 churnings with a roller-less churn, the "O.H.K." churn. The butter was judged after 3 to 4 d. at 13° C. and again after 17 to 18 d. at 13° C. It was found that when the roller-less churn was used, better results were obtained in quality, moisture distribution and yield. Working the butter in a roller-less churn resulted in a closer textured butter with less air content. With a low pH content, the greater air content aided the oxidation defects. A high pH and poor moisture distribution helped to further bacteriological defects.

G. H. Wilster

Also see abs. no. 19.

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

10. **Formula for determining the value of skim.** L. C. THOMSEN, Dept. Dairy Ind., Univ. of Wis. Nat. Butter Cheese J., 40, 11: 24-26, 51. Nov., 1949.

The value to be placed on any skim milk that is transferred to by-products is a difficult problem in cost accounting. Although a free and competitive market may be lacking for skim milk, it usually will exist for cream. With this in mind, the following formula is presented to be used in determining the value of skim milk:

$$\frac{[(A+B) - C \times F \times (D+E)] \times 100}{H} = I$$

In which

A = Amt. paid/cwt. for whole milk, f.o.b. plant.

B = Handling costs (receiving and separating) /cwt. of milk.

C = Fat in each 100 lb. of milk purchased.

D = Price paid for butterfat, f.o.b. plant (of equal quality to that received in the whole milk) in cream which may be bought.

E = Handling charge (receiving) /lb. of butterfat in cream which may be bought.

F = A constant.

H = Lb. of skim milk obtained from each 100 lb. of milk purchased.

I = Value of skim milk/cwt.

The constant "F" will be characteristic of the factory. It is the ratio of the amount of butterfat that must be purchased in the form of cream to that purchased in whole milk to produce an equivalent amount of end product, normal losses of handling both products being considered.

H. E. Calbert

11. **Body of cultured cream.** E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. Milk Plant Monthly, 38, 10: 70-73. Oct., 1949.

The body of cultured cream is largely dependent on the pasteurization temperature and homogenization pressures used in its preparation. Therefore, 18% cream should be pasteurized at 165° F. for 30 min. and homogenized at 3000 lb. pressure if a firm dry body is desired. Following homogenization, the product should be cooled to 72° F. and inoculated with a 2% starter transfer, allowing 15 hr. for ripening. The final acidity in terms of lactic acid should be 0.65% at the termination of the ripening period. The product may then be cooled to 40° F. without agitation. The final evaluation of the body of cultured cream is facilitated by the use of a plummet designed by L. D. Hilker. Plummet readings of 6 to 8 appear to be the most satisfactory from the consumer standpoint. J. A. Meiser

12. **The manufacture of quality buttermilk.** N. C. ANGEVINE, Meyer Blanke Co., St., Louis. Mo. Milk Plant Monthly, 38, 10: 26-30, 32, 35. Oct., 1949.

Only fresh skim milk of excellent quality should be used in the manufacture of cultured buttermilk. If non-fat dry milk solids are used, 8.5 lb. of a spray-processed product should be dissolved in 10 gal. of water at a temp. of 80 to 90° F. Pasteurization is then accomplished at 185° F. for 30 min. or 200° F. for 45 to 60 min. followed by cooling to 70 to 72° F. A 1% inoculation requires a ripening period of 12 to 15 hr. without agitation. This should produce a resulting acidity of 0.80 to 0.85% and signal the addition of butterfat and salt, the latter at the rate of 1 lb./100 gal. of buttermilk. The addition of butterfat in the form of granules may be accomplished by churning the vat of buttermilk at the ripening temp. with the addition of 2% fat or by adding chilled granules directly to the buttermilk when agitating. Also, dropping of melted butterfat into the chilled product is permissible. Following the ripening period, the buttermilk should be cooled to 60° F. or below, accompanied by agitation to break the curd. Storage of the bottled product should be at 45° F. for several hr. J. A. Meiser

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

13. **Professor, Dr. phil. et scient. Sigurd Orla-Jensen in memoriam.** (Professor Sigurd Orla-Jensen) M. T. SODE MOGENSEN. Nordisk Mejeri-Tidsskrift, 15, 6: 67-69. 1949.

Prof. Sigurd Orla-Jensen and his wife had returned from a trip June 11 to their home in Copenhagen, Denmark trusting that some relief might come for the threatening heart condition which caused the sudden death of Prof. Orla-Jensen on Fri., June 24, at the age of 78. He was born in Copenhagen, Nov. 28, 1870. He held a position as mechanical engineer at the Carlsberg Brewery for a few years but he became interested in dairy manufacturing and spent 2 yr. studying with Prof. Segelcke at the Royal Agricultural College. He sought further training with Weigmann in Kiel, with Duclaux at the Pasteur Institute in Paris and with von Freudenreich in Bern. He was 29 yr. of age when he was placed in charge of the dairy research experiment station at Bern, Switzerland. In 1906 he became professor of biological chemistry at Denmark's Polytechnic High School. He held this position until 1946 although from 1941 he served as a substitute for Prof. Henrik Dam who was in the U. S. at that time.

In 1919, Prof. Orla-Jensen's famous monograph, "The Lactic Acid Bacteria", was published and this brought him world renown. A supplement to it was published in 1942. His text book on dairy bacteriology was translated into English, German, Dutch, Finnish and Russian. His interest in bacteriology, microbiology and chemistry was international and he was present regularly at international dairy conventions. On one occasion he was a guest of the Argentine government and when he visited the U. S. he was popular for his articles and interviews.

At the age of 76, Prof. Orla-Jensen became interested in finding out how to maintain optimum health during the later years of life. He worked in cooperation with the Danish bacteriologist, E. Olsen, and the medical director, T. Geill, of Copenhagen. They achieved some worthwhile results through the study of diet and the part that the factors of diet played in producing certain bacteria in the intestinal flora which, in turn, influenced general health.

The final problem to which Prof. Orla-Jensen gave much attention was the curing of cheese by a quick method and in a way to bring about a characteristic desirable flavor, which would create a demand for cheese for export.

He will be missed by all who knew him. Those who saw him in the setting of his beautiful garden and summer cottage at Karlslunde know how he loved beauty. Denmark lost one of its great sons with the passing of Prof. Sigurd Orla-Jensen.

G. H. Wilster

**14. Is the methylene blue reduction test of any value?** C. K. JOHNS, Central Exptl. Farm,

Ottawa, Can. J. Milk and Food Technol., 12, 5: 267-269, 278. Sept.-Oct., 1949.

The modified inversion technique should outweigh any disadvantages formerly associated with the methylene blue reduction test because the reduction time is more comparable to the standard plate count, the dye is decolorized uniformly throughout the tube and the reduction time is shortened, especially with low count milks.

H. H. Weiser

**15. Lysogenic strains of lactic streptococci.** B. REITER. Nature, 164, 4172: 667. 1949.

A good many strains of lactic streptococci, including some commercial lactic acid cultures, were found to be lysogenic. Starters composed of strains which are lysed by the phages of lysogenic strains are unsuitable for cheese making and should be avoided.

R. Whitaker

**16. Effect of calcium on the development of streptococcal bacteriophage.** D. I. SHREW. Nature, 164, 4168: 492. 1949.

Ca stimulated phage development in 8 strains of *S. cremoris* when present to the extent of 0.001M with 0.02 to 0.007M as the optimum, depending on the media used. The Ca may be in the form of milk serum or as chloride. The addition of citrate reverses the stimulatory effect of Ca. Phage development, as measured by lysis, was not stimulated by Mg ions.

R. Whitaker

**17. The lactic acido-proteolytic bacteria and the genotypicity of the bacterial enzymes.** C. GORINI. Univ. of Milan. Enzymologia, 12, 2: 82-87. 1947.

This group of lactic acid bacteria appears to be differentiated on the basis of their reactions on proteins rather than on carbohydrates. These organisms demonstrate considerable variation in their enzymatic activities in general. This variation and dissociation may be readily reversible and the author concludes that the classification of this group will not be possible until the basic enzyme patterns of the various species are recognized.

J. J. Jezeski

**18. The ester-hydrolyzing enzyme systems of *Aspergillus niger* and of *Penicillium roqueforti*.** P. J. FODOR AND A. CHARI, Hebrew Univ., Jerusalem. Enzymologia, 13, 5: 258-267. 1949.

Monobutyryl, methyl butyrate and olive oil were used as substrates in the presence of M/5 phosphate buffer with incubation at 30-38° C. for 48 hr. Glycerol extracts of mycelia and media in which the molds had grown were used as

sources of the enzymes. Free fatty acids were estimated by titration. On the basis of pH optimum studies, evidence was obtained which suggested that 2 types of esterases were produced by each of the molds. An intracellular enzyme extracted from the mycelia had an optimum at pH 6.5 (or below), while the enzyme present in the culture medium had an optimum about pH 8.0. Sodium taurocholate inhibited both types of enzymes. J. J. Jezeski

19. Die Bedeutung der Wasserstoffionenkonzentration bei der Konservierung von Nahrungsmitteln und Viehfutter. (The significance of hydrogen ion concentration in the preservation of foods and feeds.) English summary. A. I. VIRTANEN. Die Milchwissenschaft, 3, 12: 353-361. Dec., 1948.

Microbial growth is limited, in general, to the pH range of 1.0 to 13.0. Proteins are not readily attacked below pH 4.0. Thus, if silage is adjusted to between pH 3.0 to 4.0 it readily can be kept from spoilage.

Butter is stored best at pH 6.0 to 7.0. At this pH level the formation of fishy and oily flavors is prevented. Bacterial spoilage at this pH must, however, be prevented by proper pasteurization of cream and sanitary butter manufacture.

Animal products, such as fish meal, meat meal, etc. can be preserved by the addition of slaked lime to pH 12.0 and by storage in air-tight containers. The oxidation-reduction potential, similar to the pH, plays an important part in the preservation of all types of nutrients.

I. Peters

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

20. Lipolytic flavors of milk. N. P. TARASSUK AND E. L. JACK, Univ. of Cal., Davis. Milk Plant Monthly, 38, 10: 48. Oct., 1949.

Lipolytic flavors produced by the action of lipase on milk fat is accelerated by disruption or distortion of the absorbed fat globule layer through homogenization, shaking or heat shock of raw milk. Also, certain milks are naturally lipolytically active and will develop spontaneously a rancid flavor. In the latter case, it is necessary that the milk be cooled before lipolysis begins which suggests that lipase is present in the milk serum prior to cooling.

J. A. Meiser

21. The "sunlight" flavor in milk. D. G. KEENEY, Penn. State College. Milk Plant Monthly, 38, 10: 54. Oct., 1949.

A sunlight flavor may be produced artificially by the addition of 25 to 75 p.p.m. of formaldehyde to milk flashed to 167° F. or above. Colorimetric determination of the amount of formaldehyde reacting in the milk was less than the sensitivity of the method used. Although the dialyzable portion of the skim milk appears to promote this flavor, exposure of the diffusate to sunlight did not result in a flavor development.

J. A. Meiser

22. Separation and estimation of saturated  $C_2$ - $C_8$  fatty acids by buffered partition columns. VIVIEN MOYLE, E. BALDWIN AND R. SCARISBRICK, Univ. of Cambridge. Biochem. J., 43, 2: 308-317. 1948.

A chromatographic method of separating the lower molecular wt. naturally occurring fatty acids is given. Phosphate-buffered silica gels are used as the solid phase and mixtures of chloroform and n-butanol as the solvents. Reported recoveries of fatty acids in various mixtures range from 97 to 103%. A. O. Call

23. The mechanism of fatty acid oxidation. L. F. LELOIR, Fundac. Campomar, Buenos Aires. Enzymologia, 12, 4: 263-276. 1949.

Critical papers are reviewed dealing with fatty acid oxidation in animal tissues and oxidation by microorganisms. The results are interpreted on the basis that a reactive two-carbon compound is formed as the result of beta-oxidation of the fatty acids, as well as from pyruvate oxidation. Thus, the final stages of both fatty acid and carbohydrate oxidation may have a common pathway. J. J. Jezeski

24. Liberation of amino acids from raw and heated casein by acid and enzyme hydrolysis. L. V. HANKES, W. H. RIESEN, L. M. HENDERSON AND C. A. ELVEHJEM, Univ. of Wis., Madison. J. Biol. Chem., 176, 2: 467-476. Nov., 1948.

Previous work showed soy bean protein was affected adversely by heat. This investigation shows that autoclaving casein at 15 lb. pressure for 4 min. did not affect the ease with which the amino acids were liberated and made microbiologically available. The only ill effects from a 20 hr. period was a reduction in the cystine availability. A table shows the percentage of the various amino acids liberated from the raw and heat-treated casein as measured after acid hydrolysis (alkaline for tryptophan and tyrosine) and enzymatic digestion. A. O. Call

25. Amino acid composition of  $\alpha$ -casein and  $\beta$ -casein. W. G. GORDON, W. F. SEMMETT, R.

S. CABLE AND M. MORRIS, Eastern Regional Research Lab., Philadelphia, Pa. J. Am. Chem. Soc., 71, 10: 3293-3297. Oct., 1949.

The amino acid composition of whole casein and its 2 major components,  $\alpha$ -casein and  $\beta$ -casein, was determined. Essentially all of the nitrogen of each protein was accounted for in terms of known amino acid residues and amide nitrogen. Most striking differences in  $\alpha$ -casein and  $\beta$ -casein were observed in proline, tryptophane, cystine and tryosine content. Histidine, glutamic acid, threonine and amide nitrogen were considered to be present in equal concentration. The small differences in glycine, isoleucine and serine content are believed to be significant.

$\beta$ -casein exhibited a greater solubility in ethanol-water mixtures, probably due to the larger proportion of non-polar groups. The amino acid composition also may explain the differences in electrophoretic mobility of  $\alpha$ -casein and  $\beta$ -casein. In solutions both acid and alkaline to the isoelectric points of the proteins,  $\alpha$ -casein had a higher mobility. The higher proportions of cationic and anionic groups in  $\alpha$ -casein would explain this observation.

H. J. Peppler

26. The chemical composition of acropeptides from casein and their behaviour towards enzymes. A. FODOR, P. J. FODOR AND S. KUK-MEIRI, Hebrew Univ., Jerusalem. Enzymologia, 12, 2: 101-106. 1947.

Acropeptides, nonhydrolytic protein derivatives which do not represent open chains of amino acids and therefore do not possess terminal amino or carboxyl groups, were prepared by dissolving casein in water-free glycerol at 135-145° C. and then precipitating in alcohol. The smallest unit obtained appeared to be an octapeptide and a larger one was studied which contained 9 such units. From data obtained on amino acid composition, elementary analysis and presence of  $\text{NH}_2$  and free  $\text{COOH}$  groups (side chains) it has been concluded that the casein molecule is composed of these amino acid complexes held together by associative linkages.

J. J. Jezeski

27. A comparative study of the behavior of proteins and acropeptides towards proteinases. P. J. FODOR, S. KUK-MEIRI AND A. FODOR, Hebrew Univ., Jerusalem. Enzymologia, 12, 2: 107-113. 1947.

The relative action of pepsin-HCl and a pancreatic proteinase toward casein and an acropeptide presumably containing 72 amino acid units was studied. The amount of cleavage

caused by the action of pepsin on the acropeptide was proportional to the number of free carboxyl groups and that caused by the pancreatic enzyme was proportional to the number of free  $\text{NH}_2$  groups. This acropeptide was similar to casein in its behavior toward the hydrolytic enzymes studied.

J. J. Jezeski

28. De formoltitratie; een practijkmethode voor de bepaling van het gehalte van koemelk aan "totaal" eiwit en aan caseïne. (The formol titration; a practical method for the determination of total protein and casein in cow's milk.) (English summary.) H. J. BANNENBERG AND W. VAN DEN HOEK, College of Agriculture, Wageningen, Holland. Netherlands Milk & Dairy J., 3, 3: 162-177. July-Sept., 1949.

The formol number  $w$  as determined in 21 milk samples without using potassium oxalate and total protein and casein content also were determined. A conversion factor was calculated by dividing the determined formol titre by the percentage protein (or casein). This conversion factor was  $1.011 \pm 0.038$  ( $r = +0.55$ ) for total protein and  $0.807 \pm 0.034$  ( $r = +0.44$ ) for casein; the greatest possible deviations from the true values were 10 and 12%, respectively. Another experiment was run the same way with 28 samples of milk using the Pyne modification with potassium oxalate. Here the conversion factor for total protein was  $0.350 \pm 0.005$  ( $r = +0.94$ ), and for casein,  $0.278 \pm 0.004$  ( $r = +0.93$ ); the greatest possible deviations from the true values being 4.3% in both cases. Composite samples were used in the previous experiments. The influence of the stage of lactation was checked by examining 15 milk samples of single cows in different stages of lactation using the Pyne method. These conversion factors agreed with the composite milk values, except for the last 2 mo. of lactation which gave a somewhat higher total protein value ( $0.361 \pm 0.011$ ), while the first 4 d. of lactation gave unreliable results. The milk acidity changed the formol titre only when the milk no longer was acceptable for manufacturing purposes. The conclusion was that the Pyne method can be used for determining the protein and casein content with sufficient accuracy.

A. F. Tamsma

Also see abs. no. 43.

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

29. Flow diversion valve. E. C. HARTMAN (assignor to Taylor Instrument Co.). U. S. Patent 2,484,622. 4 claims. Oct. 11, 1949. Official Gaz. U. S. Pat. Office, 627, 2: 528. 1949.

A flow diversion valve for high temperature, short time pasteurizers is described. The forward flow outlet valve assembly is equipped with a leak-detecting opening to prevent the passage of under pasteurized milk while the valve is in the diverted position.  
R. Whitaker

**30. Het vraagstuk van de afvalwateren der zuivelindustrie.** (Waste liquids in the dairy industry.) (English summary.) J. H. A. SCHAAFSMA, State Dairy Consultant. Netherlands Milk & Dairy J., 3, 2: 142-154. April-June, 1949.

Methods of treatment and disposal of dairy waste waters, the present state of affairs and prospects and possibilities in the Netherlands are discussed. Since 1920 the Government Institute for Purification of Waste Waters was mainly in charge of this work for the dairy industry.

The varying character and composition of dairy waste and consideration of local circumstances make it impossible to give a general procedure for purification. First of all, disposal of organic material caused by losses in the manufacturing process and loss of by-products should be reduced to a minimum. The remaining waste liquid should be purified by some oxidative method which is cheap and efficient. Most methods, differing technically, are based on the same microbiological oxidation process of organic material by oxygen of the air. Difficulties can be caused by too high concentration or too low pH caused by lactic acid bacteria. The waste liquid must be kept fresh; sometimes lime or chlorine can help here. In the Netherlands most dairy factories are satisfactory, while some isolated cases still are unsatisfactory. Due to a lack of proper legislation these cannot be forced to change procedures. Without too much cost there is an opportunity for improvement through proper investigation by a sanitary adviser and using the facilities available. Further research to establish more economical purification systems and international interchange of results and ideas should be strongly encouraged and organized.  
A. F. Tamsma

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**31. Cost—Each individual product in the ice cream plant.** E. J. MATHER, National Dairy Products Corp. Ice Cream Trade J., 45: 10, 62. Oct., 1949.

Key personnel should know the cost of each flavor in bulk ice cream and the cost of each product, such as: bulk, pt. package, qt. package,

0.5 gal. and 1 gal. packages, slices, molds and other products, including the cost of each novelty. To arrive at the price to dealers, manufacturing, selling, administration and other cost factors should be known.

To arrive at a correct delivery cost per product, the space occupied by the unit of sale of each product can be related in terms of points to a gal. of bulk ice cream as a base unit with a point value of 1. A table can be established for all products with point values related to bulk ice cream according to the space occupied.

One of the best ways to reduce cost in the plant is to employ better men, or give those already employed a better education so they will be in a position to find and use the methods that will produce the best products at the lowest possible price.  
W. H. Martin

Also see abs. no. 10.

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**32. Availability of the magnesium of grass to the ruminant.** R. J. GARNER. Nature, 164, 4167: 458. 1949.

Mg is not made available by the action of the gastric juice of ruminants, but is liberated from vegetable cells by the ruminal organisms. Free Mg does not exist in the alkaline rumen but becomes available by the action of the abomasal HCl.  
R. Whitaker

**33. The fermentation of cellulose in vitro by organisms from the rumen of sheep.** H. R. MARSTON, Univ. of Adelaide, S. Australia. Biochem. J., 42, 4: 564-574. 1948.

An apparatus intended to simulate the conditions found in the rumen is described. Water suspensions of cellulose, from birch bark in 2 cases and from filter paper in 2 cases, to which inorganic salts were added and then inoculated with a "community of microorganisms" from the rumen contents of sheep, were fermented. The predominate products of dissimulation were acetic and propionic acids, CO<sub>2</sub> and methane. Smaller quantities of formic, butyric, pyruvic and lactic acids as well as acetaldehyde were reported. The energy metabolism of the ruminant is discussed in view of these findings.  
A. O. Call

**34. The nutritive value for the calf of colostrum and its fractions.** (Abs.) R. ASCHAFFENBURG et al., Natl. Inst. for Research in Dairying, Univ. of Reading and Research Inst. of Animal Path., Royal Veterinary College, Biochem. J., 42, 2: xxx-xxxi. 1948.

Both Ayrshire and Shorthorn colostrum were separated with a super-centrifuge into fatty and non-fatty fractions. Various combinations of these 2 fractions were made with dried skim milk and margarine and then fed to a total of 66 calves. Best results were obtained with the untreated colostrum group, followed by the group fed the non-fatty fraction. The essential factor in the aqueous phase of colostrum is concluded to be active in small concentrations. The vitamin A reserves at 5 wk. were not related to the initial intake.

A. O. Call

Also see abs. no. 7, 46.

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

35. **Superovulation and ovum transfer in cattle.** R. E. UMBAUGH, Foundation of Applied Research, San Antonio, Texas. *Am. J. Vet. Research*, 10, 37: 395-305. Oct., 1949.

Superovulation was induced in cows by implanting a pellet containing 1500 to 3000 r. u. of pituitary gonadotrophin followed in 4 d. by intravenous injection of 500 to 1500 r. u. of pituitary gonadotrophin or by daily subcutaneous injection of 50 to 500 r. u. pituitary gonadotrophin for 5 d. followed by intravenous injection of 25 to 1000 r. u. pituitary gonadotrophin. Pregnant mare serum gonadotrophin and chorionic gonadotrophin were not effective for superovulation. Ova were collected at the time of spontaneous ovulation, 26 hr. following the intravenous injection, by aspirating the follicles with capillary pipettes via a flank incision. Percentage recovery of ova and percentage fertilization were reduced in the high-level hormone treatments. Treatment with stilbestrol or progesterone did not change the fertilization ratio. Following superovulation an average of 23.4 ovulation points/cow were counted and 10.4 ova/cow were collected from the oviducts, but only 5.8/cow were fertilized. Sixteen fertilized ova were transferred to the fallopian tube of 1 cow the day after estrus, and 17 ova were transferred to another. Multiple embryos developed but were aborted prematurely.

E. W. Swanson

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

36. **Supporting arrangement for milkers.** H. B. BABSON (assignor to Babson Bros. Co.). U. S. Patent 2,483,516. 8 claims. Oct. 4, 1949. *Official Gaz. U. S. Pat. Office*, 627, 1: 134 1949.

A frame surrounding a milking stall is described having a moveable arm, counterbalanced by a weight, to support a milking machine vessel.

R. Whitaker

37. **Teat cup.** L. DINESEN. U. S. Patent 2,484,696. 1 claim. Oct. 11, 1949. *Official Gaz. U. S. Pat. Office*, 627, 2: 548. 1949.

This teat cup for milking machines has a rigid outer shell and a flexible inner tube. The space thus formed is connected to a source of pulsating vacuum.

R. Whitaker

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

38. **Double seal milk can.** J. A. HOPWOOD. U. S. Patent 2,484,624. 5 claims. Oct. 11, 1949. *Official Gaz. U. S. Pat. Office*, 627, 2: 528. 1949.

To provide additional protection, the cover of this milk can seals in 2 places. The cover not only fits snugly into the throat of the can, but the outside rim of the flared-can-top engages a skirt attached to the inside of the can lid.

R. Whitaker

39. **Gladsaxe Mejeri, et af Storkobenhavns mest moderne Mejerier. (Gladsaxe Mejeri—One of Greater Copenhagen's Most Modern Dairies.)** *Nordisk Mejeri-Tidsskrift*, 13, 6-7: 57, 58. 1947.

The Gladsaxe dairy plant presents an exterior which is typical of a large, remodeled milk plant. Upon coming into the building it is apparent at once that the interior is equipped so as to be technically and hygienically up to date and efficient. Working conditions are good, with ample room for all of the necessary operations but with a working plan that eliminates the waste of time and effort.

A large bottle washing machine is in use and when the bottles have been washed, rinsed and sterilized they travel in cases, through an opening in the wall, directly to the bottle-filling machine. From there, the filled bottles travel on a track, on which the cases of filled bottles roll into the cooling room.

The milk as it is received and weighed into tanks is handled in the same efficient, time- and labor-saving manner. It goes through the separator and into a plate pasteurizer. From there it goes into aluminum tanks on a balcony and it is agitated by the use of air under pressure.

The large vacuum filling machine has 16 filling outlets. In the bottom of each milk tank are pipes which are connected with the vacuum filling machine. There is no way of making errors and having buttermilk flow into the bottles intended for fresh milk or vice versa. Everything is done accurately and hygienically. The cases of bottled, cooled milk travel on rollers into a large milk storage room that has a capacity of 20,000 l. This room is cooled by an Atlas cool-



ing apparatus. The cases then travel out to the milk delivery platform where the milk trucks wait to receive them.

Large modern storage rooms are provided for dairy products and supplies. A large basement is equipped with a fresh-water tank and a brine tank, as well as with a lunch room, dressing room and wash room for workers in this modern plant. A floor plan accompanies the article.

G. H. Wilster

Also see abs. no. 14, 20, 21, 29.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**40. Synthesis of the short-chain fatty acids of milk fat from acetate.** G. POJÁK, S. J. FOLLEY AND T. H. FRENCH, Univ. Reading, England. Arch. Biochem., 23, 3: 508-510. Oct., 1949.

The probability that the short-chain acids of milk fat ( $C_4$ - $C_{14}$ ) originate from acetate has been indicated by results obtained *in vitro* with mammary gland slices from ruminants as well as non-ruminants. Pregnant rabbits injected with  $CH_3C^{14}OONa$  exhibited a high rate of incorporation of  $C^{14}$  into glyceride fatty acids extracted from the mammae. Fractionation of these fatty acids revealed that the volatile acids contained 7-18 times more isotope than the non-volatile acids. The highest  $C^{14}$  content was found in the water-soluble fraction comprised chiefly of butyric and caproic acids. The low  $C^{14}$ -content of unfractionated fatty acids of the liver indicates that this organ is not the source of the highly active fatty acids in the mammae. H. J. Peppler

Also see abs. no. 41, 42.

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**41. The effect of thyroxine on the metabolism of lactating cows. 1. General results and nitrogen metabolism.** E. C. OWEN, The Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., 43, 2: 235-243. 1948.

Eight Ayrshire cows were used in an investigation to determine the effect of subcutaneous injection of 10 mg. of thyroxine/d./cow. The cows were subjected to 3 different levels of feed intake. Thyroxine increased catabolism, as shown by an increase in pulse rate. This resulted in an increase in milk yield, a loss in body wt., an increase in the urine excretion and a negative nitrogen balance. The negative nitrogen balances were inhibited somewhat by an increase in feed. The composition of the milk during thyroxine administrations showed an increase in fat and solids

as well as an increase in nitrogen in the fat-free portion, as compared with that of the controls.

A. O. Call

**42. The effect of thyroxine on the metabolism of lactating cows. 2. Calcium and phosphorus metabolism.** E. C. OWEN, The Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., 43, 2: 243-247. 1948.

In connection with thyroxine studies (see previous abstract) Ca balances were made on 8 Ayrshire cows. In all but 1 case the Ca balances were negative before, during and following the administration of thyroxine; however, in 2 cases where the cows were fed more liberally, the output of Ca was increased by giving thyroxine.

P balances were made on only 2 cows. They both showed positive balances throughout the experiment and giving thyroxine tended to increase the amount retained. The P content of the milk was increased significantly by thyroxine but the Ca content was unaffected.

A. O. Call

**43. Preparation of radioactive iodocasein.** C. F. HAMILTON, MARSCHELLE H. POWER AND A. ALBERT, Mayo Clinic and Mayo Foundation, Rochester, Minn. J. Biol. Chem., 178, 1: 213-216. March, 1949.

A procedure, following in general the method of Reineke and Turner, is given for the preparation of iodocasein containing radioactive iodine ( $I^{131}$ ). It is intended for human metabolism studies.

A. O. Call

**44. The effects of large doses of various sulfonamides injected intravenously in dairy cattle.** L. M. JONES, H. A. SMITH AND M. H. ROEPKE, Iowa State College, Ames. Am. J. Vet. Research, 10, 37: 318-326. Oct., 1949.

The sulfonamides sulfathiazole, sulfapyridine, sulfadiazine, sulfamerazine, sulfamethazine and sulfaquinoxaline were each injected into separate groups of cows as the sodium salt dissolved in 500 cc. of water. One series of cows received 1 injection of 60 g. of each sulfonamide. Another series received 2 60-g. injections 7 to 10 d. apart, and a 3rd series of cows was given the same plus 2 90-g. injections. Blood concentrations were maintained best by sulfamethazine, followed in order by sulfapyridine, sulfamerazine, sulfadiazine, sulfaquinoxaline and sulfathiazole. Sulfaquinoxaline was very toxic, resulting in the death of 1 cow following the first injection, and was not used for higher level studies. The other sulfonamides also caused weakness and collapse of some of the cows but recovery was rapid. One cow given the highest dosage of sulfapyridine died from hemorrhage. Livers and kidneys of

all cows showed damage. Extensive myelin degeneration of sections of the spinal cord and of the sciatic and median nerves was found in paralyzed cows following sulfaquinolaxaline injection. Body temperature and blood urea were not varied from normal. E. W. Swanson

**45. Studies on the gross anatomy of the bovine liver. I. The distribution of the blood vessels and bile ducts as revealed by the vinylite-corrosion technique.** L. M. JULIAN AND K. B. DE-OME, Univ. of Cal., Berkeley. *Am. J. Vet. Research*, 10, 37: 331-335. Oct., 1949.

Different colored vinylite solutions were injected into the vascular systems and bile ducts of the liver and a study made of the subgross anatomy. Portal circuits clearly were demonstrated. Numerous arterial plexuses were found around the bile ducts. The article is well illustrated.

E. W. Swanson

**46. The absorption of ammonia from the rumen of the sheep.** I. W. McDONALD, *Biochem. Lab., Univ. of Cambridge. Biochem. J.*, 42, 4: 584-587. 1948.

The general circulation blood of sheep contains very little, if any, ammonia, while about 1.5 mg. ammonia N/100 ml. is present in the venous blood which comes from the rumen. A nitrogen cycle in the digestive tract is postulated, wherein ammonia from the rumen is converted in the liver to urea, which may be secreted in the saliva and returned to the rumen where it is again converted to ammonia. A. O. Call

**47. Amino acid composition of  $\beta$ -lactoglobulin and bovine serum albumin.** W. H. STEIN AND S. MOORE, Rockefeller Inst. for Medical Research, N. Y. *J. Biol. Chem.*, 178, 1: 79-91. March, 1949.

Chromatographic fractionations of the amino acids of  $\beta$ -lactoglobulin and bovine serum albumin hydrolysates have been made using starch columns and a mixture of n-butyl alcohol, n-propyl alcohol and HCl as the solvent. Recoveries of about 98% of the amino acids on a wt. basis are reported. The results are in good agreement with previously reported values.

A. O. Call

Also see abs. no. 6, 23, 35.

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**48. Some new quaternary ammonium compounds and their properties.** J. C. L. RESUOGAN, F.R.I.C. Director and Chief Chemist, The

British Hydrological Corp. *Dairy Inds.*, 14, 8: 819-822. Aug., 1949.

Some new twin-chain quaternary ammonium compounds are described with regard to solubility, bactericidal properties and compatibility with certain anionic and non-ionic compounds. The results show that with the twin-chain compounds a greater degree of solubility could be attained without sacrificing much bactericidal activity. Didecylmethylammonium bromide, having a total of 20 carbon atoms in its 2 chains, is more soluble than with a single long chain compound containing 20 carbon atoms. With greater degree of solubility there is less tendency for precipitates to be formed with other compounds.

In testing quaternary ammonium compounds for bactericidal properties, each quaternary compound must be recognized as a special case and the proper inhibitor selected to prevent bacteriostatic effect.

G. H. Watrous, Jr.

**49. Laboratory evaluation of cleaner-sanitizers for use on dairy farms.** F. W. BARBER, National Dairy Research Lab., Inc., Oakdale, L. I., N. Y. *J. Milk and Food Technol.*, 12, 5: 257-266. Sept.-Oct., 1949.

A heat resistant culture of *E. coli*, known to be resistant to the action of quaternary ammonium compounds, was added to a cleaner-sanitizer test solution. A known concentration of ice cream mix was added to supply the organic matter comparable to farm conditions in cleaning dairy utensils. The study included variable factors such as temperature changes, degree of water hardness and organic matter as they may effect the bactericidal action of quaternary ammonium compounds. The technique approximates the claims made by manufacturers of sanitizing compounds used in actual practice.

H. H. Weiser

**50. Effectiveness of hypochlorite and quaternary ammonium compounds in a mastitis sanitation procedure.** K. R. SPURGEON, W. J. HARPER AND P. R. ELLIKER, Purdue Agr. Expt. Sta., W. Lafayette, Ind. *Milk Plant Monthly*, 38, 10: 42-46. Oct., 1949.

To determine the effectiveness of hypochlorites and quaternary ammonium compounds in destroying mastitic organisms, milking machine teat cups were inoculated with *S. agalactiae* by swabbing with a milk suspension of the organism. After inoculation, the cups were rinsed a predetermined number of times in varying concentrations of the germicides under study and allowed to drain for periods ranging from 30 sec. to 5 min. Actual counts of the surviving organisms were obtained by inoculating cold, sterile skim

milk which in turn was plated on veal infusion blood agar. Of the hypochlorites and quaternary ammonium compounds selected for the final study, none destroyed all of the *S. agalactiae* but the reduction in numbers suggested the application of these compounds in milking procedures where teat cups are dipped between cows. Factors affecting the efficiency of these compounds in destroying the test organism were the use of prolonged exposures, the use of two successive rinses and the use of increasing concentrations of the germicide. The high numbers of surviving organisms on rubber teat cup inflations that possess cracks or checks emphasized the inadequacy of

germicidal treatment when worn out or improper equipment is used on the farm. J. A. Meiser

**51. Tiermedizinische Milchhygiene im Rahmen des Reichsmilchgesetzes.** (Veterinary milk hygiene in the scope of the government milk law.) English summary. E. PAARMANN. *Die Milchwissenschaft*, 3, 12: 371-372. Dec., 1948.

Considering the financial losses in cattle and in beef as a result of bovine tuberculosis and also the health hazards to the milk-consuming public, the author strongly suggests that a systematic obligatory irradiation program of tuberculin-infected cattle should be started in Germany.

I. Peters

#### *PUBLICATIONS OF THE XII INTERNATIONAL DAIRY CONGRESS.*

In conjunction with the XII International Dairy Congress in Stockholm, August 1949, the dairy specialists of 40 countries submitted more than 400 reports containing much data of scientific and practical value. These papers were printed in 5 volumes. A sixth volume contains General Reports, prepared by eminent specialists who have summarized each of the 33 different subjects taken up for discussion at the Congress. A further volume, no. 7, will comprise a report on the actual Congress and a record of the oral debates. This volume is now being edited and will be released in 1950. Further particulars are given below.

<i>Volume no.</i>	<i>Subject</i>	<i>No. of papers</i>	<i>Pages</i>
1	Milk production, hygiene and control, tropical dairying	86	552
2	Physics, chemistry and microbiology	126	766
3	Dairy technique	88	566
4	Economy and trade, organization of the dairy industry	82	694
5	Supplement containing further papers on the 6 subjects given above	27	200
		<hr/> 409	<hr/> 2,778
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# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**52. The control of *Streptococcus agalactiae* infection in herds by means of therapeutic treatment.** S. J. EDWARDS and J. I. TAYLOR. Vet. Record, 61, 47: 780-783. 1949.

One herd of 70 Ayrshire cows that had been tested regularly for *Str. agalactiae* infection for several years and was machine milked and stripped, with precautions taken to prevent the spread of mastitis, was studied first. Just prior to treatment, 54 cows were shown to be infected 22 showing clinical signs of mastitis. Infected cows were treated for 4 d. with 50 ml. of a 30% sulphanilamide emulsion in an oil-water base per quarter, and 61% of the infected cows responded to this treatment. Another treatment was given 2 wk. later using up to 8 daily injections if daily samples indicated infection was still present. Sulphanilamide finally was effective in 85% of infected cows treated. Remaining infected cows plus newly infected cows were treated with penicillin in water, using 4 daily injections of 100,000 units each per quarter. This treatment was successful and, with the exception of 3 new cases, the herd remained free of *Str. agalactiae* infection for over 2 yr.

Five more herds infected with *Str. agalactiae* were included, in which the incidence of infection was 21%. Treatment consisted of 4 daily injections of 40,000 units of penicillin in a 4.5% beeswax-oil base. In the first course of treatment, 31 of the 55 infected cows were treated and 24 served as controls. All but 2 of the treated cows responded, and in subsequent treatment of the 24 controls in which infection remained, all but one responded. Herds remained in the experiment for 20 mo., and in 2 herds the infection was eradicated, while in a 3rd herd it was reduced to a single cow that did not respond to successive treatments. In the 2 remaining herds the incidence was lowered for a short period, but rose repeatedly. Teat lesions and no precautions to

prevent the spread of infection were believed to account for this.

The authors concluded that some cows infected with *Str. agalactiae* resist treatment from both sulphanilamide and penicillin, with penicillin being most effective. R. P. Niedermeier

**53. A comparison of ante-mortem and post-mortem findings in bovine mastitis.** D. McFARLANE and P. S. BLACKBURN. Vet. Record, 61, 49: 807-810. 1949.

This experiment was designed to obtain a comparison between the diagnostic value of cell count and culture tests and to determine whether typical mastitis pathogens are present in quarters producing milk of high cell content but free of pathogens. Fifty-four quarters with a record of high cell counts were examined and ante- and post-mortem data are presented. Cell counts of an animal were considered positive if the average count of the mid-lactation samples was over 100,000/ml. Post-mortem histological results were considered positive if there was evidence of what the authors term progressive or dormant mastitis. Pathological findings were present in 92% of the quarters with high cell counts, indicating that cell counts are a reliable method of diagnosis for mastitis.

In 39 quarters, a comparison was made between ante-mortem milk cultures and post-mortem udder tissue cultures, and 80% agreement was obtained. Further comparisons resulted in 56% agreement between ante-mortem culture tests and post-mortem tissue culture tests and histological examination. In 39% of the histologically positive quarters no typical mastitis organisms were found in the milk ante-mortem or in the tissues post-mortem, indicating a mastitic condition can exist even when culture tests are negative.

The authors conclude that culture tests of the milk are not as reliable for diagnostic work as cell count tests, and a non-specific form of bovine mastitis often may be present, although mastitis organisms or other pathogens are not present in the milk or udder tissues. R. P. Niedermeier

**54. The agglutination reaction of bovine serum in the diagnosis of trichomoniasis.** A. E. PIERCE, Ministry of Agriculture, Veterinary Labs., Weybridge, England. *British Vet. J.* 105, 8: 286-294. Aug., 1949.

A large series of sera from normal and trichomonad infected cattle was checked with the agglutination test for aid in the diagnosis of bovine trichomoniasis (early abortion). Specific agglutinins to *Trichomonas foetus* were detected in the sera from known trichomonad infected herds. The test is regarded as a herd test. All tests must be carried out using 2 different strains of trichomonads. Of 179 samples, checked in duplicate, from infected herds 15 showed positive, 8 above normal, 17 slightly above negative and 139 negative. Animals showing a titer for *Brucella abortus* did not react to the trichomonas agglutination test.

B. B. Morgan

**55. Sulfamerazine and sodium sulfamerazine as therapeutic agents in cattle.** R. H. WALKER, Pleasanton, Cal. and E. V. EDMONDS, Oakland, Cal. *Vet. Med.*, 44, 10: 415-417. Oct., 1949.

Fourteen of 16 adult cattle infected with foot rot responded satisfactorily to a single dose of 20-48 g. of sodium sulfamerazine given intravenously (12 animals) or intraperitoneally (2 animals). Twenty-three of 30 cases of several other infections also responded satisfactorily to 1 intravenous injection of an aqueous solution of sodium sulfamerazine at a dosage of 3-4 g./100 lb. body weight. The diseases included 7 cases of diphtheria, 7 cases of pneumonia, 4 cases of shipping fever, 4 cases actinobacillosis and 1 case each of tendonitis, infection of mandible, streptococcal infection of the jaw, sinusitis, septicemia (mastitis), necrotic vaginitis, infected wound and necrophorus skin infection. No toxic reactions were observed after infections of the drug in cattle.

B. B. Morgan

**56. Quinoline diphosphate in experimental anaplasmosis.** E. J. SPLITTER, Kansas State College, Manhattan. *Vet. Med.*, 44, 10: 418-419. Oct., 1949.

In a very limited experiment 2 splenectomized calves experimentally infected with *Anaplasma marginale* were treated either with quinoline diphosphate or quinoline phosphate. Neither drug showed any specific action against anaplasmosis.

B. B. Morgan

**57. Cattle grub distribution in California.** D. P. FURMAN, J. R. DOUGLAS and K. G. MCKAY,

Univ. of Cal., Berkeley and Davis. *J. Econ. Entomol.*, 42, 5: 842-843. Oct., 1949.

Cattle grub collections from native cattle were made in 44 Cal. counties over a 2-yr. period. Both *Hypoderma bovis* (northern cattle grub) and *H. lineatum* (common cattle grub) were found in 34 counties. Each of the 2 species was found alone in 5 counties. *H. bovis* was distributed from northernmost to southernmost Cal. Presence of *H. bovis* in Imperial County, which borders Mexico, is thought to be its southernmost incidence in the U. S.

*H. lineatum* appears in cattle backs earlier than *H. bovis*, and is replaced by the latter species late in the season. Relative abundance of both species is not constant in all areas of the state. Where both are numerous, grubs emerge from cattle backs during a period of over 6 mo. In such cases 3 grub treatments at 30-d. intervals will not give adequate control.

E. H. Fisher

**58. An attempt to protect cattle from grub infestation by use of insecticides.** O. H. GRAHAM, U.S.D.A., Bureau of Entomology and Plant Quarantine. *J. Econ. Entomol.*, 42, 5: 837. Oct., 1949.

Near Kerrville, Texas, cattle were sprayed each 2 wk., from Jan. 1 to Apr. 28, for common cattle grub control. The grub flies were active during this period. Wettable powders of DDT, DDD (TDE), methoxychlor, chlordan and toxaphene were used at 2% concentration. BHC at 0.24% gamma isomer plus 1.76% other isomers and a combination of 0.75% DDT and 0.03% gamma BHC plus 0.22% other isomers were included. Each cow's entire body was wet to the skin with 5 gal. of spray/animal, applied at 300 lb. pressure. All materials were applied 9 times, except chlordan; 3 of 10 cows sprayed with chlordan died after the 4th application.

Results were obtained by determining the numbers of grubs in the gullets and backs of cattle at various periods. No treatment completely protected cattle from infestation. The 0.24% gamma-BHC treatment gave best control; however, this was a high concentration of the insecticide, and this material is not considered of great practical value for grub control if applied at safe concentrations only 2 or 3 times during the season of adult (fly) activity.

E. H. Fisher

**59. County-wide control of the horn fly with DDT.** C. L. SMITH and D. E. GATES, U.S.D.A., Bureau of Entomology and Plant Quarantine. *J. Econ. Entomol.*, 42, 5: 847-848. Oct., 1949.

During the 1948 horn fly season, a county-wide horn fly control program was carried out in Kiowa County, Kan. The test plot covered 720 sq. mi. on which were about 25,000 head of cattle, including both beef and dairy breeds, and many farm buildings which needed treatment. Less than 1% of about 650 farm owners failed to co-operate in the cattle spraying. About 80-85% of the buildings were treated. Initial cattle sprays were applied between Apr. 26 and May 25. Retreatment was made when the flies averaged 25/animal.

Cattle spray was 0.5% DDT, made from a 50% DDT wettable powder. This was applied at 450-500 lb. pressure, using about 1 gal./animal. Building spray was 5% DDT, made from 25% DDT emulsifiable solution, applied at 75-100 lb. pressure.

Eradication of the horn fly from a large area was shown to be difficult, and 100% community effort is necessary for a successful program of this type. Data are presented. E. H. Fisher

**60. Suspected buttercup poisoning in a Jersey cow.** O. V. GUNNING, Acle, Nr., Norwich. British Vet. J., 105, 10: 393. Oct., 1949.

A case history on a suspected buttercup poisoning in a Jersey cow is given. All of the symptoms pointed to poisoning, as the pasture was full of buttercups in the flowering stage. B. B. Morgan

## BUTTER

O. F. HUNZIKER, SECTION EDITOR

**61. Treating butterfat.** C. E. NORTH. U. S. Patent 2,485,308. 6 claims. Oct. 18, 1949. Official Gaz. U. S. Pat. Office, 627, 3: 811. 1949.

The flavor of rancid butterfat may be improved by emulsifying the objectionable fat with skim milk powder and water at a temperature about that at which the fat melts, to form a cream of about 35-40% fat. After cooling the remade cream is churned and the fat recovered.

R. Whitaker

**62. Butter cutter.** M. A. BERG. U. S. Patent 2,488,656. 1 claim. Nov. 22, 1949. Official Gaz. U. S. Pat. Office, 628, 4: 1045. 1949.

Pats of butter are ejected from this butter cutter after they are cut by a heated blade from a block of butter stored in a chilled condition on a frame above the cutter. R. Whitaker

**63. Cream sediment tester.** N. C. KOTTKAMP and P. J. BAILEY (assignors to Langenkamp-Wheeler Brass Work, Inc.) U. S. Patent 2,487,248. 7 claims. Nov. 8, 1949. Official Gaz. U. S. Pat. Office, 628, 2: 422. 1949.

A tubular tester for inserting into cans of cream, the volume of product to be tested being controlled by finger-operated air valves in the top and 2 attached float valves which open and close depending on the volume desired.

R. Whitaker

Also see abs. no. 74, 78, 79, 86, 91.

## CHEESE

A. C. DAHLBERG, SECTION EDITOR

**64. Rasprostrannost streptokokkovo bakteriofaga saragh.** (The incidence of streptococcal bacteriophage in cheese.) E. B. RUNOW. Mikrobiologia, 8: 174-176. 1949.

Sixty-seven Russian domestic cheese varying in age from 2-48 mo. were examined for the presence of bacteriophage. The cheese made from raw or pasteurized milk included the following types: Jaroslav, Dutch square, Dutch round, Gouda, Volga, Uglitsk, Tilsit, Soviet, Camembert, Cheddar and Swiss.

To 20-30 g. of ground cheese was added water at 45° C. in amounts to get a ratio of cheese to water of 1:2.5. The cheese-water mixture was stored in sterile containers for 18-20 hr. at 4-10° C. Cheese with acidities below the precipitation point of the protein were acidified with lactic acid. The mixture was filtered through filter paper followed by a Zeiss filter which filtrations rendered the filtrate clear in most instances. Aseptic methods were used throughout.

One-ml. portions of the various filtrates were added to 9-ml. quantities of sterile milk containing 1 loopful of streptococcus culture sensitive to bacteriophage. A control tube without added filtrate also was prepared. All tubes were held at 35° C. for 12 hr. and examined microscopically for evidence of bacterial lysis and acid development.

By giving to the amount of acid produced by the control tube an arbitrary value of 100%, 14.7% of the filtrates retarded acid production by 30-40%, 8.8% by 40-50%, 16.2% by 50-60%, 30.9% by 60-70% and 29.4 by over 70%. The conclusion was reached that bacteriophage active against lactic streptococci is widely distributed among cheese and apparently is a permanent factor in the surroundings of cheese. I. Peters

**65. Kyllagringsförsök med Ost (Low-temperature storage experiments with cheese.)** K. E. THOMÉ, T. BERGMAN and S. HOFF. Svenska Mejeriernas Riksförening Meddelande No. 7. In yearly report Alnarp Lantbruks-Mejeri-och Trädgårsinstitut, pp. 84-144. 1948.

At the Swedish Dairies Association's Riksst cold storage plant at Växjö, about 3,500 cheeses

were used for these experiments. The types of cheese were Herrgård, Gouda and Svecia. A part of the cheese was stored at  $-2^{\circ}\text{C}$ . and part of it was stored at the normal storage temperature of  $13-14^{\circ}\text{C}$ .

The experiments were divided into 3 sections: (a) cheese stored at normal temperature, placed in storage at different ages, (b) to determine how cheese from the same lot reacts to different combinations of normal and low temperature storage, (c) intended to answer questions regarding the low-temperature technique and economy.

The usual rules (*i.e.* 1-10 points for size and shape, surface and rind, color, texture, consistency, taste and flavor) were used for grading the cheese. Six experienced judges did the grading. The degree of ripening was determined according to protein decomposition (Mogensen's method) and volatile acids present.

Nothing in the experiments pointed to any abnormal activity with regard to protein-decomposition during low-temperature storage. It was retarded, however, by the low temperature. The formation of volatile acids seemed to stop altogether during low-temperature storage and it did not restart after the storage temperature was raised. It seemed that the volatile acids formed during the production of the cheese and during the very first part of the storage period was the only volatile acid that had any effect on the grade of the cheese. The average age of the cheese suited for the experiments was between 2 and 3 mo. when placed in storage. If the cheese is stored at such an age as to be fully ripened when removed from storage, it follows that it will become over-ripe if left for any length of time at normal temperatures.

Experiments were made to determine how the cheese should be handled during storage at a low temperature. All of the cheese when first placed in storage was checked for condition of surface and rind. Re-treatment with paraffin during 6-mo. storage did not appear to be necessary if the cheese had reached a suitable age and degree of ripening. For these tests 3-4 mo.-old Herrgård and 1.5-3 mo.-old Svecia and Gouda cheese were used.

During low-temperature storage when placed separately on the shelves, normal Svecia cheese needed no turning. It was found best to turn Herrgård and Gouda cheese every month or every second month, however, to prevent the upper side from becoming sunken.

At normal temperature storage at a temperature of  $+13^{\circ}\text{C}$ ., it was regarded as the best plan to turn the cheese every third day in the early part of the storage period but less often toward the end of the storage period. Paraffin treatment of cheese stored at the normal storage tem-

perature seemed to be necessary about once a month.

Svecia cheese can be stored on edge but in order to avoid damage to the rind, the cheese should be rotated slightly at least once a week. This storage method makes better use of available space. A photograph in the bulletin illustrates how storage of cheese on edge may increase the storage capacity by about 67%.

If the cheese is stored in piles of not more than three cheese in each pile, no damage seemed to result to the cheese and the storage capacity was increased by 67%. It further was indicated that if some of the shelves that had been removed when piling the cheese 3 deep, could be assembled in the hallways, the storage capacity could be increased by 150%. Although these experiments were carried out on a small scale, the pile-storage method was adopted by the Riksst Association after the experiments were completed. It was used successfully both for Svecia and Herrgård cheese.

The cost of storing cheese for 6 mo. at normal storage temperature compared with storing it for 6mo. at the low temperature, showed the low-temperature storage cost was 50% lower than that for normal temperature storage.

After the completion of the experiments the low-temperature storage cheese was, as a whole, of better quality than the cheese stored at the normal temperature.

Low-temperature storage seemed to improve the consistency of the cheese, but the greatest advantage in this method was in the balancing of the seasonal variations in manufacture. Next in importance was the greater utilization of storage space during the summer when storage space is in great demand.

G. H. Wilster

66. **Curd cutting apparatus.** E. C. DAMROW (assignor to Damrow Bros. Co.) U. S. Patent 2,488,053. 3 claims. Nov. 15, 1949. Official Gaz. U. S. Pat. Office, 628, 3: 762. 1949.

Several forking arms, pivoted to a motor and gear assembly mounted above the cheese vat, are caused to rotate. The lower ends of the arms, bent parallel to the bottom of the cheese vat, are equipped with teeth for stirring the curd.

R. Whitaker

67. **Citric acid esters in cheese.** C. M. GOODING, R. H. NEAL and H. W. VAHLTEICH (assignors to Best Foods Co.) U. S. Patent 2,485,637. 20 claims. Oct. 25, 1949. Official Gaz. U. S. Pat. Office, 627, 4: 1031. 1949.

Mono- and di-alkyl and -alkylene esters of citric acid are used as antioxidants in cheese products

comprising butterfat, non-fat milk solids and milk proteins.

R. Whitaker

**68. Cheese cutting machine.** P. J. SCHLUDE. U. S. Patent 2,489,504. 1 claim. Nov. 29, 1949. Official Gaz. U. S. Pat. Office, 628, 5: 1396. 1949.

A device is described for cutting cheese into small pieces suitable for wrapping for retail trade.

R. Whitaker

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**69. Skyr—Islands nasjonalrett. (Curds—Iceland's national dish.)** OLAV KLOKK. Meieri-posten, 38, 30: 526-528. July, 1949.

Skyr is a nourishing, tasty food which keeps well and which can be transported unchanged, for long distances. It has been reported that if the product is stored in wooden kegs, it will remain palatable for several years. The directions for making it are as follows:

The skimmed milk must first be warmed to 90° C. and then quickly cooled to 40° C. In warm weather it is cooled to 30° C. To 200 kg. of milk, 15 g. of rennet should be added (strength of rennet was not given) and 2 l. of skyr from a former batch. When no skyr from a former batch is available to use, it may be necessary to repeat the curd-making process a few times before the desired results are obtained. The milk, with rennet added, must be kept evenly warm, 40-50° C. for 2 hr. or longer, depending upon the rennet action. The coagulated milk is poured on a strainer cloth stretched over a wooden frame. The whey is drained off and a weight of 6-8 kg. may be placed on the curd to press it. When the whey has drained off, the soft curdy mass is packed in parchment-lined wooden kegs holding 60-70 kg.

One kg. of skyr is made from 3 kg. skimmed milk. In Reykjavik, during harvest season, 1 kg. of skyr cost kr. 3.50. A liter of whole milk brought kr. 1.90 and the price for cream was kr. 14.60/l.

G. H. Wilster

**70. Stabilizing evaporated milk.** H. E. OTTING (assignor to M and R Dietetic Laboratories, Inc.) U. S. Patent 2,490,599. 4 claims. Dec. 6, 1949. Official Gaz. U. S. Pat. Office, 629, 1: 248. 1949.

By means of a base exchange treatment, milk is prepared having a Ca:P ratio of 0.815 to 1.155. To stabilize evaporated milk, from 8-60%, based

on total solids, of the treated milk is added, prior to sterilization in the range from 220-270° F.

R. Whitaker

**71. Milk evaporator.** A. W. BAUMANN. U. S. Patent 2,485,689. 6 claims. Oct. 25, 1949. Official Gaz. U. S. Pat. Office, 627, 4: 1044. 1949.

The novel feature of this vacuum pan for condensing milk is the method of introducing the milk into the pan. The insert pipe passes down through the middle of the coils to within a short distance of the bottom of the pan. The end of the pipe is flanged outwardly. The milk forced between the flange and bottom of the pan flows outwardly and upwardly around the coils.

R. Whitaker

**72. Non-starch dessert composition.** F. GATTI (assignor to G. Fabre.) U. S. Patent 2,485,043. 4 claims. Oct. 18, 1949. Official Gaz. U. S. Pat. Office, 627, 3: 748. 1949.

A mixture of agar-agar, dextrose and NaHCO<sub>3</sub>, when added to milk to form a dessert, does not curdle the milk. The amount of dextrose exceeds the agar-agar and the proportion of NaHCO<sub>3</sub> may be from 15-40/100 parts agar-agar.

R. Whitaker

**73. Amino acid compositions and their preparations.** D. B. HAND, J. G. BRERETON and O. W. KAUFMAN (assignors to Sheffield Farms, Inc.) U. S. Patent 2,489,880. 4 claims. Nov. 29, 1949. Official Gaz. U. S. Pat. Office, 628, 5: 1494. 1949.

Acid whey at pH 4.5 is heated to 140° F. to coagulate the proteins. The coagulated proteins, dispersed in water at pH 6.8-7.2, are digested by the proteolytic enzymes in macerated pancreas for several days at 110-115° F. The enzymes are inactivated by heat, any solid material filtered off and the soluble amino acids concentrated.

R. Whitaker

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**74. Water-insoluble fatty acids and butyric acid in cream and butter.** F. HILLIG, H. A. LEPPER and W. I. PATTERSON, Food and Drug Administration, FSA, Washington 25, D. C. J. Assoc. Offic. Agr. Chemists, 32, 4: 731-735. 1949.

Cream was held for various periods of time and determinations made for titrable acidity, butyric acid, propionic acid, lactose and water-insoluble acids. Experiments show that as cream ages and decomposes, the fat may break down form-



ing water-insoluble acids. In some cases, quantities of water-insoluble acids far in excess of those normally present in sweet cream were observed. The results suggest that water separators may be one of the factors causing early decomposition of cream. Data are presented on the water-insoluble acids in 321 samples of commercial butters. Butyric acid was present in some of the butters containing the larger quantities of water-insoluble acids. Analyses on numerous cans of cream that actually were used in commercial churnings showed variations in water-insoluble acids ranging from 50 to 6000 mg./100 g. F. J. Babel

**75. Detecting foreign fats in ice cream.** W. H. MARTIN, W. D. RUTZ and C. H. WHITNAH, Kansas State College, Manhattan. *Ice Cream Trade J.*, 45, 11: 48-49, 85-88. Nov., 1949.

Reichert-Meissl, Polenske and Kirschner numbers were determined on fat extracted from ice cream and from ice cream in which 5 different vegetable fats had been substituted for one-third of the butterfat. The Reichert-Meissl number measures the volatile, soluble fatty acids of fats and oils, the Polenske number measures the insoluble, volatile fatty acids and the Kirschner number approximates the butyric acid content of the fats.

Butterfat from cows fed normal rations has a Reichert-Meissl value ranging from 24 to 33, while most other fats and oils have numbers of 1 or less. Coconut oil, with a Reichert-Meissl number of 7, is an exception. Polenske numbers of butterfat vary between 1.5 and 3 and coconut oil has a Polenske number between 16.8 and 17.8. Other fats and oils generally have values of less than 1. If ice cream contains foreign fats in addition to butterfat, the Reichert-Meissl number of the resulting mixture should be the weighted average of these fats. Data presented show that this relationship existed. W. H. Martin

**76. Refractive indices of lactose solutions.** F. W. ZERBAN and J. MARTIN, N. Y. Sugar Trade Lab., New York, N. Y. *J. Assoc. Offic. Agr. Chemists*, 32, 4: 709-713. 1949.

The refractive indices of lactose hydrate solutions, containing up to 32% of this sugar, were determined at 20° C. to the 5th decimal place with a Bausch and Lomb precision refractometer. The results obtained agree satisfactorily with those reported by McDonald (*J. Research Nat. Bur. Standards*, 41: 63. 1938). Results obtained by previous authors were compared with the more recent ones and critically discussed. F. J. Babel

**77. Stabilization of standard carotene solutions.** M. L. COOLEY, General Mills, Inc., Larro Research Farm Lab., Rossford, O. *J. Assoc. Offic. Agr. Chemists*, 32, 4: 706-709. 1949.

Small quantities of mixed tocopherols or pure  $\alpha$ -tocopherol in petroleum ether solutions of purified carotene provide an antioxidant effect by which carotene is preserved from deterioration for as long as 12 wk. Without tocopherols, destruction of carotene was pronounced in 2 or 3 d. To effect proper stabilization, from 10 to 50 times as much tocopherols as carotene was necessary. Use of more than 5 mg. of tocopherols/100 ml. of petroleum ether was not advisable because of the production of a measurable amount of color. F. J. Babel

**78. Stabilized fats and oils.** J. KORNER (assignor to Selmo Chemical Corp.) U. S. Patent, 2,486,177. 1 claim. Oct. 25, 1949. Official Gaz. U. S. Pat. Office, 627, 4: 1170. 1949.

Butter and other edible fats and oils are protected against oxidation by an antioxidant consisting of ammonium gallate or a substituted ammonium gallate. R. Whitaker

**79. Margarine and butter composition.** H. W. VAHLREICH, R. H. NEAL and C. M. GOODING. (assignors to Best Foods Co.) U. S. Patent 2,485,634 12 claims. Oct. 25, 1949. Official Gaz. U. S. Pat. Office, 627, 4: 1030. 1949.

Dialkyl and dialkylene esters of citric acid are used as antioxidants for margarine and butter at the rate of 0.5-1.5%. R. Whitaker

**80. Praktiska försök över ljusets inverkan på mjölkens smak under olika betingelser. (Practical experiments to determine the influence of light upon the flavor of milk under varying conditions.)** HILMER DANIELSSON, Svenska Mejeritekniska Meddelande, no. 2: 3, 8. 1947.

By covering some transparent glass milk bottles with dark-colored cardboard cartons and then leaving an equal number of milk bottles uncovered, it was possible to gain an understanding of the effect of light upon milk. All of the bottles of milk in the experiment were placed together and held at the same temperature for the same length of time. After 0.5 hr. the milk was judged and again after a 24-hr. storage period at 10° C. Milk in bottles covered with dark-colored paper cartons did not develop the oxidized or metallic flavor defect.

By treating milk with hydroquinone, it was impossible to prevent the development of the flavor defect to some extent. This was true especially when exposing milk to direct sunlight. Brown glass bottles were useful in retarding the develop-

ment of an oxidized or metallic flavor in milk. After 1 hr. of exposure to light, only a slight change was noticed in the flavor of the milk. After storing for 24 hr., milk in brown glass bottles which had been exposed to direct sunlight for 30 min. showed only a very slight change in flavor.

It is advisable to keep milk and milk products protected from light, and especially from sunlight.

G. H. Wilster

**81. Nonenzymatic browning of foodstuffs. Production of carbon dioxide.** V. M. LEWIS, W. B. ESSELEN, JR., and C. R. FELLERS, Univ. of Mass., Amherst. Ind. Eng. Chem., **41**, 11: 2587-2591. Nov., 1949.

Production of CO<sub>2</sub> in a variety of foodstuffs was studied by sealing the samples in pyrex tubes and analyzing the headspace gases for CO<sub>2</sub> after long storage periods. Many foods generated CO<sub>2</sub> on storage at 100° C., Cheddar cheese producing 1.14 and 1.80 mg/g. after 3 and 7 d., respectively. A study of the reaction between reducing sugars and amino acids with regard to CO<sub>2</sub> production showed that decarboxylation of the amino acid was an integral part of the reaction at 100° C.

B. H. Webb

**82. Nonenzymatic browning of foodstuffs. Nitrogen-free carboxylic acids in the browning reaction.** V. M. LEWIS, W. B. ESSELEN, JR., and C. R. FELLERS, Univ. of Mass., Amherst. Ind. Eng. Chem., **41**, 11: 2591-2594. Nov., 1949.

A reaction that produced browning of foods was found to occur between glucose and the carboxylic acids in general. The effect of pH, temperature and oxygen on the glucose-carboxylic acid reaction, particularly that involving citrates, lactates and acetates was determined. The reaction was inhibited completely by SO<sub>2</sub> in the absence of oxygen when the pH of the medium was low. The color produced by the carboxylic acid-glucose reaction is of the same order as that produced by the amino acid-glucose reaction. It is believed that the nitrogen free acids play an important role in the browning of foods.

B. H. Webb

**83. Production of protein filaments.** W. A. CALDWELL and E. R. WINTON (assignors to Imperial Chemical Industries.) U. S. Patent 2,489,519. 8 claims. Nov. 29, 1949. Official Gaz. U. S. Pat. Office, **628**, 5: 1401. 1949.

An aqueous solution of a protein such as casein is extruded into acid saline coagulating bath and the resulting filaments stretched at a temperature of not over 40° C. in a saline solution which has

no swelling effect. The stretched fibre then is passed through a hot saline solution at 40° C. or above, again causing no swelling of the filament.

R. Whitaker

**84. An ultraviolet spectrophotometric method for the quantitative estimation of benzene hexachloride in milk.** J. P. FRAWLEY and B. DAVIDOW, Food and Drug Administration, FSA, Washington 25, D. C. J. Assoc. Offic. Agr. Chemists, **32**, 4: 758-762. 1949.

The method is based on the dehydrohalogenation of benzene hexachloride to 1,2,4-trichlorobenzene and the estimation of the latter compound by means of an ultraviolet spectrophotometer. The method is applicable to all the isomers, and is sensitive to 0.1 mg. of benzene hexachloride. As outlined, the method will quantitatively estimate 0.5 p.p.m. in milk.

F. J. Babel

Also see abs. no. 70.

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**85. Elastic guide for cream separator shafts.** E. C. J. JADOU. U. S. Patent 2,488,295. 18 claims. Nov. 15, 1949. Official Gaz. U. S. Pat. Office, **628**, 3: 827. 1949.

Four rigid guides arranged equidistantly about the shaft of a cream separator are held in place by elastic members which urge the shaft to rotate without vibration in case of misbalancing.

R. Whitaker

**86. Centrifuge with primary and secondary zones of separation and process therefor.** I. J. LUNDAL (assignor to Sugar Creek Creamery Co. and Cherry Burrell Corp.) U. S. Patent 2,485,209. 22 claims. Oct. 18, 1949. Official Gaz. U. S. Pat. Office, **627**, 3: 787. 1949.

This milk or cream separator bowl is designed to discharge continuously 3 components, (a) cream, (b) skim milk and (c) precipitated protein, foreign matter, slime and other materials of high density. The product to be separated enters a pre-separating chamber, where the high density materials are discharged through a port to the outer edge of the bowl and thence through another port in the bowl wall; the cream and skim milk, unseparated, pass through a large opening into a second chamber. The second chamber, containing cone-shaped discs, is essentially the same as the conventional cream separator and the cream and skim milk are separated and collected in the usual manner.

R. Whitaker

**87. Pasteurizing and cooling apparatus.** W. J. MILLER. U. S. Patent 2,489,043. 2 claims. Nov. 22, 1949. Official Gaz. U. S. Pat. Office, 628, 4: 1147 1949.

Milk and other fluids are heated and cooled by following a spiral track through this heat exchanger which consists of horizontal disks held in place by a vertical frame. R. Whitaker

**88. Governor.** W. H. HARSTICK (assignor to International Harvester Co.) U. S. Patent 2,484,995. 4 claims. Oct. 18, 1949. Official Gaz. U. S. Pat. Office, 627, 3: 735. 1949.

This governor, designed to control the speed of a vertical, series-wound, motor-driven cream separator, depends for its action on the air pressure built up by an impellor operating in a housing on the bottom of the motor. R. Whitaker

**89. Don't fool with ammonia systems.** W. DAVIS, Natl. Safety Council. Operating Engineer, 2, 10: 44-45. Oct., 1949.

Periodical maintenance of equipment and safety devices cuts the accidents. Safety valves may be tested by a simple out-of-the system method which makes use of a high pressure grease gun with proper line and gauge fittings. The piping for this set-up is illustrated. Heat exchangers eliminate the danger of slugs of ammonia returning to the compressor. When possible, stop the compressor while tightening packing glands. Packing glands should be tightened to eliminate ammonia leaks. Oil separator discharge lines should run to a closed vessel containing water. Operators have been severely burned by ammonia being discharged into open buckets or vessels.

Air in the system mixing with lubricating oil is dangerous. Never use oxygen at high pressure to test refrigeration system. Oxygen-lubricating oil mixtures may ignite without a spark. Do not tighten pipe joints until the line is free of pressure. Leaks indicate some weakness; when a nut is tightened, the bolt may break and, if the line is under high pressure, cause the joint to break. Pipe line insulation must be kept in good condition. Broken insulation may permit condensation on the pipe which in turn leads to corrosion.

H. L. Mitten, Jr.

**90. Milk cooler use of heat removed from milk.** R. C. SHIPMAN, United Coop. Labs., Ithaca, N. Y. Agr. Eng., 30, 11: 531-532. Nov., 1949.

A number of 4-can milk coolers were tested using procedures of the American Society of Refrigerating Engineers Standard Methods of Rating and Testing Complete Can-Type Milk Coolers.

Cooling rate curves are presented which indicate that: (1) agitation of the water bath assists materially in moving heat from can to water, (2) agitation of water bath aids in reducing temperature difference between top and bottom of can, (3) the greatest amount of heat is removed from the can during the 1st hr. in the cooler, (4) if water contacting can is 40° F. or below 1st hr., warmest part of can will be below 50° F. within 1 hr. and (5) only small can temperature differences exist in all types of coolers at end of a 12-hr. period.

Fast cooling at time of loading is dependent upon the amount of stored refrigeration and the maintenance of cooling medium temperature below 40° F. Total running time and power required/degree temperature change was very similar for all the coolers even though 3 were powered by 0.33 hp. motors and 2 by 0.25 hp. motors.

In considering the use of heat from milk to warm the milk house, it is recognized that heat is removed from milk in a shorter time in winter than in summer and that less milk is produced in winter. An additional heat load can be placed in the cooler to increase its operating time by adding water. Water should be added in such a manner as to keep the cooling water under 40° F. and not to destroy the required stored refrigeration.

H. L. Mitten, Jr.

**91. A low-cost mechanical cooler for holding cream.** H. L. MITTEN, F. E. SATCHELL, J. J. McDOW, and A. W. FARRALL, Mich. State College, E. Lansing. Agr. Eng., 30, 11: 525-527. Nov., 1949.

A low-cost, dry-box, mechanical cooler is described. This cooler was designed, built and tested at Michigan State College. On-the-farm tests indicated that the mechanical cooler offered a positive means of maintaining cream quality at low operating costs. Drawings are presented which show dimensions, cooling oil arrangement and insulating details.

H. L. Mitten, Jr.

**92. Deaerating heater licks corrosion in creamery.** E. W. F. FELLER, Operating Engineering, Albany 1, N. Y. Opr. Engineering, 2, 11: 24-25. Nov., 1949.

Corrosion in heaters and condensate return lines and traps caused by dissolved oxygen and CO<sub>2</sub> in feedwater necessitated frequent pipe replacement in a Cal. creamery. Oxygen content of the feedwater was reduced to 20% of its former quantity by replacing the open feedwater heater with a deaerating unit. Great savings in maintenance resulted. Every ammonia receiver and evaporator in the creamery is piped to a manifold outside the plant so that any unit may

be dumped to the sewer in case of fire. The manifold valves are encased in glass.

H. L. Mitten, Jr.

**93. Treat rotary pumps right.** A. M. SHAW, Worthington Pump and Machinery Corp., Harrison, N. J. *Operating Engineer*, 2, 11: 38-39. Nov., 1949.

Because rotary pumps are built to close clearances, they require careful handling and installation to insure long, trouble-free life.

External strains on pump casing or drive shaft may cause much damage. The pump must be carefully aligned with its driver to prevent the transmission of radial and axial thrusts to the pump and piping should be supported as near pump as possible to prevent distorting strains. The suction line piping should never be smaller than the pump opening. If thick liquids are to be handled, the suction piping may be larger than the pump opening. The suction line should be short and direct. The pump should not be run dry, for it will wear rapidly or seize. Mechanical seals must be tight. Seal faces may be reconditioned by holding the seal flat and moving its face in figure eights over fine lapping paper which is resting on a flat plate or piece of plate glass.

H. L. Mitten, Jr.

**94. How lubrication licks friction.** Anonymous. *Power*, 93, 10: 98-99. Oct., 1949.

Friction is classified as sliding, rolling and fluid. Lubrication is a means of separating parts to eliminate sliding friction and substituting rolling friction or fluid friction. The types of friction and elementary ideas of lubrication are presented pictorially. The lubrication engineer produces lubricants to fit certain requirements. He blends mineral oils, compounds mineral oils with vegetable or animal fats to make a lubricant for various conditions of pressure, temperature and moisture.

Lubricating oils are of three types: animal, vegetable and mineral. Animal oils are generally used in compounds of animal and mineral oils. Tallow, lard oil and degas are used. Tallow is used in white greases and as a saponified base to hold lubricating oils in grease. Lard oil is used for cutting oils in lubricants and in stainless oils. Of the vegetable oils, castor oil is the best lubricant. It does not mix well with mineral oil unless another fixed oil is present.

Mineral oils are made from crude petroleum. There are several large oil-producing regions in the U. S. Pennsylvania fields produce paraffin-base crudes; mid-continent fields produce mixed-based; and western (and most foreign) fields produce asphalt-base crudes. Most lubricating oils

are blended of various crude stocks available to gain a preferred group of properties.

Solid lubricants consist of graphite, talc, soapstone, flowers of sulphur, white lead and similar materials.

Refining processes are discussed.

H. L. Mitten, Jr.

**95. Quick check for pipe stress, thrust.** J. J. Blank. *Power*, 93, 10: 87-89. Oct., 1949.

Equations, tables and curves are presented for the rapid determination of allowable pipe stress and thrust. Tables, curves and instructions are complete and can be applied without explanation. The tables are based on the principle of the "guided cantilever". Examples of application also are presented.

H. L. Mitten, Jr.

**96. Humidifiers—how to select and apply.** T. G. Hicks, New York, N. Y. *Opr. Engineering*, 2, 11: 34-35. Nov., 1949.

Before a humidifier can be chosen, it is necessary to know (1) the relative humidity desired, (2) indoor temperature desired, (3) minimum recorded outdoor temperature for plant locality and (4) the number of air changes needed/hr.

Humidifiers are classed as direct and indirect. Direct humidification is used where high humidities must be held and cooling and ventilation are not important. Indirect humidification is used for comfort air conditioning. A combination of indirect and direct is used in industrial application where cooling or ventilating loads are high. Direct humidifiers are identified as (1) atomizing, (2) high-duty, (3) spray and (4) self-contained or centrifugal. Indirect humidifiers use a heated coil to vaporize water and a fan to distribute the vapor.

An example illustrates the method of selecting a humidifier. Several drawings and a moisture-humidity curve are presented.

H. L. Mitten, Jr.

**97. Careful planning produces training program that clicks.** E. W. F. FELLER, *Power*, 330 W. 42nd St., New York, N. Y. *Power*, 93, 11: 74-77. Nov., 1949.

The power plant training program of Armstrong Cork Co. is described. It provides information on how to (1) prepare a boiler for service, (2) fire with different fuels and (3) handle turbines. Its aim is to teach each operator (1) what he *must* know and (2) what he *should* know. Sound slide films in color are used. Topics covered are steam generation, feedwater treatment and operating rules for boilers. Subjects such as valve construction, flue-gas analysis

and steam accessories are taught with large posters. Each student is required to answer a 2-page folder of questions. In addition to the films and posters, operating procedures are provided for every piece of power equipment in each plant. This permits on-the-job training on specific items of equipment.

A "Leaders' Guide" is issued each power-plant foreman. It contains instructions on (1) how to handle a meeting, (2) how to set up, adjust and operate the projector and (3) what to tell the group before running the film. It is claimed that outlay for the training courses has been repaid many times in more efficient operation, suggestions for improvements and better employee relations.

H. L. Mitten, Jr.

Also see abs. no. 71.

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**98. Concentrations of various constituents in blood of dairy cows during stages of terminal gestation and initial lactation. I. Effect of prepartal diet on serum tocopherols.** C. E. LAT-SCHAR, G. H. WISE, D. B. PARRISH and J. S. HUGHES Kan. Agr. Expt. Sta., Manhattan. J. Nutrition, **38**, 4: 503-516. Aug., 1949.

The cows were fed typical barn rations, supplemented and unsupplemented. There was a gradual decline in blood serum tocopherol concentrations during the last month of gestation, which became more pronounced within a few days of parturition. Minimum concentrations occurred 2 d. *post partum*, after which there was an increase.

The additions of 0.5-1 g. of tocopherols to the prepartum diet resulted in serum concentrations of tocopherols of more than 15% greater than in control animals. Supplements of 4-10 g. daily gave an additional increase in serum tocopherols. These larger supplements, however, did not prevent the decline during the parturient period. Amounts of tocopherols in the blood serum of 1 cow which was milked throughout gestation remained fairly constant during the parturient period.

R. K. Waugh

**99. Determination of sugar in forage plants.** J. W. THOMAS, C. G. MELIN, and L. A. MOORE, Bur. Dairy Ind., U.S.D.A., Washington, D. C. Analyt. Chem., **21**, 11: 1363-1365. Nov., 1949.

A rapid method is described for extracting forage plants, including green and dried plant materials, by mixing them 5 to 7 min. in a Waring Blendor. Carotene and sugar were extracted simultaneously from green forage ma-

terials by using a mixture of ethanol and Skellysolve in the blender. When aqueous NaCl was added to the extract, carotene remained in the epiphase while the hypophase was used for sugar analysis. It was not necessary to clarify the extract with lead. Extraction with the Waring Blendor was as complete as with the longer A.O.A.C. method of Soxhlet extraction.

H. B. Webb

Also see abs. no. 77.

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

**100. A seminal defect associated with sterility of Guernsey bulls.** J. L. HANCOCK and D. H. L. ROLLINSON. Vet. Record, **61**, 45: 742-743. 1949.

A morphological abnormality of spermatozoa found in 12 young Guernsey bulls with a breeding history of total sterility is described. Photomicrographs show an absence of intact spermatozoa, with only free heads and tails present; no sample examined had more than 5% intact spermatozoa. This characteristic feature was observed in 73 ejaculates studied from the 12 bulls, including repeated samplings for periods of 1-8 mo. Motility ratings on the semen gave low values, and microscopic examination of diluted semen at the time of collection indicated the free tails and heads already were present, with the free tails having some movement. Motility was maintained for periods of 36-116 hr. when the semen was stored at 4° C. Density values ranged from 100,000-900,000 sperms/ml. In stained slides of the separated heads and tails, the heads appear normal with a deep indentation at the point of separation, but most of the tails are abnormal. Pedigree examination of the 12 bulls indicates the abnormality is not genetic origin nor could any other common environmental factor be found.

R. P. Niedermeier

**101. Why cows fail to conceive when bred by artificial insemination.** G. T. EASLEY, Sulphur, Okla. Vet. Med., **44**, 11: 455-459. Nov., 1949.

A general review paper gives data on the factors influencing conception (health, season, breed), factors affecting the production of normal semen (exercise, condition, frequency of use, number of ejaculates collected, age, cleaning the bull), collection and preservation of semen (semen collection, processing semen, method of insemination) and factors related to the cow (time of insemination, estrous cycle, calving interval, age, exercise, excitement, abnormal anatomical structures, infections of the reproductive and endocrine balance).

B. B. Morgan

**102. "Bulldog head" cattle.** R. B. BECKER and P. T. DIX ARNOLD, Florida Agr. Expt. Sta. *J. Heredity*, **40**, 10: 282-286. Oct. 1949.

Five of the several cases of viable prognathism (bulldog head, undershot jaw) recorded in grade Jersey cattle were specifically indicated in a 5-generation pedigree. The report is illustrated by photographs of 2 prognathous cows and normal close relatives. The side and front view of the skulls of 1 affected and 1 normal cow of the same breed also are shown. Skulls in prognathous animals were visibly wider than the normal skull. The difference in the length of the frontal bones between the 2 skulls was slight, indicating the shortened prognathous skull is caused largely by shorter maxilla and premaxilla. A shortened nasal bone dimension of 3.4 in. was noted. The orbits were larger and more nearly rectangular than in the normal. Impaired vision was associated with prognathism in the herd studied. The inheritance was concluded to be that of a single autosomal recessive gene. L. O. Gilmore

**103. An acardiac monster from a cow.** C. W. OTTAWAY, Cambridge Univ. *British Vet. J.*, **105**, 8: 318-320. Aug., 1949.

After artificial insemination from a Shorthorn bull, a non-pedigreed, Shorthorn cow with a history of 3 previous normal calvings, expelled a monster which was within the fetal membranes of a normal calf. The monster was a spherical mass covered with hair. It weighed 440 g. with a circumference of 35 cm. A complete anatomical description including dissection data is presented. B. B. Morgan

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**104. An estimate of the quarterly calving rate of heifers in Welsh counties and the percentage annual replacements in the principality. Part I. Calving rates.** R. PHILLIPS, University College of Wales, Aberystwyth. *British Vet. J.*, **105**, 9: 351-369. Sept., 1949.

After a study of returns of the quarterly rates of calving it was found that the seasonal rates of calving had shifted and that the percentage of heifers calving had increased. It was estimated that over 50% of the heifers calve during Sept. to Dec. Complete protocols are presented. B. B. Morgan

**105. An estimate of the quarterly calving rate of heifers in Welsh counties and the percentage**

**annual replacements in the principality. Part II. Estimated herd replacements.** R. PHILLIPS, University College of Wales, Aberystwyth. *British Vet. J.*, **105**, 10: 384-392. Oct., 1949.

Further studies on returns of the quarterly rates of calving showed that in the Welsh dairying counties only 1 heifer calf is reared for every 3 cows. About 4 heifers need to be reared in order to obtain 3 of them in-calf, although most counties require 3 reared heifers to get 2 in-calf.

B. B. Morgan

**106. Pulsator for milking machines.** L. DINESEN (assignor to Perfection Manufacturing Corp.) U. S. Patent 2,489,563. 9 claims. Nov. 29, 1949. *Official Gaz. U. S. Pat. Office*, **628**, 5: 1411. 1949.

A device, operated by fluid pressure, produces pulsations suitable for a milking machine by means of 2 reciprocating pistons.

R. Whitaker

**107. Milking machine time determiner.** G. T. WILLSON (assignor to DeLaval Separator Co.) U. S. Patent 2,488,754. 8 claims. Nov. 22, 1949. *Official Gaz. U. S. Pat. Office*, **628**, 4: 1071. 1949.

The rapidity of the pulsations of a milking machine are adjustably controlled by this device which electrically operates a pneumatic valve controlling the vacuum supply. R. Whitaker

**108. Milker releaser.** F. G. HODSDON (assignor to International Harvester Co.) U. S. Patent 2,488,725. *Official Gaz. U. S. Pat. Office*, **628**, 4: 1063. 1949.

Milk is released from the reduced pressure used in milking machines, from this vessel consisting of 2 chambers operated alternately by a system of valves to continuously permit withdrawal of milk. R. Whitaker

**109. Milk strainer.** D. O. BRANT. U. S. Patent 2,483,000. 4 claims. Sept. 27, 1949. *Official Gaz. U. S. Pat. Office*, **626**, 4: 1059. 1949.

This strainer is designed for use on farms for filtering freshly drawn milk into milk cans. The filtering medium is supported over the drainage outlet by several finger-like members attached to the bottom of the tapered strainer vessel. The drainage outlet consists of a tube of relatively small bore for a strainer of this type.

R. Whitaker

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**110. Promoting soft ice cream as a gallonage builder.** Anonymous. *Ice Cream Rev.*, **33**, 4: 38, 69. Nov., 1949.

The introduction of a low butterfat (6%) ice cream served direct from the freezer by the McGrath Ice Cream Co. of St. Louis, Mo., has proved to be an effective means of increasing their total sales volume during the past year. Although the introduction of soft ice cream into their 3 company-owned stores has taken some sales away from regular ice cream, the product has also stimulated the sale of carry-out packages of regular ice cream. The net result has been an increase in total sales in 1949 as compared with 1948. The soft ice cream now accounts for about 50% of their total volume. The product is sold under the name, "Frosty Kream" in the form of cones, sundaes or malts.

W. J. Caulfield

**111. Stabilized ice cream mixes.** A. B. STEINER and G. D. SPERRY (assignors to Kelco Co.) U. S. Patent 2,485,935. 4 claims. Oct. 25, 1949. Official Gaz. U. S. Pat. Office, **627**, 4: 1105. 1949.

Alginic acid is esterified by at least 40% to form a propylene glycol ester and used as a stabilizer for ice cream mix. R. Whitaker

**112. Alginate ice cream stabilizing composition.** A. B. STEINER (assigned to Kelco Co.) U. S. Patent 2,485,934. 6 claims. Oct. 25, 1949. Official Gaz. U. S. Pat. Office, **627**, 4: 1105. 1949.

An ice cream stabilizer is composed of from 40-90% of a water soluble salt of a partially depolymerized, low-viscosity alginic acid.

R. Whitaker

**113. Packaged sundae.** F. T. MOSER (assigned to Limpert Bros. Inc.) U. S. Patent 2,486,194. 4 claims. Oct. 25, 1949. Official Gaz. U. S. Pat. Office, **627**, 4: 1174. 1949.

Ice cream is filled into a paper cup in such a way as to leave a depression on the middle of the top surface into which the fruit, syrup, etc. is placed. The ice cream, surrounding the portion of topping, is in contact with the lid and prevents seepage of said topping at the edges of the lid.

R. Whitaker

**114. Packages, prices, profits—Part Two.** V. M. RABUFFO. *Ice Cream Trade J.*, **45**, 11: 56-57, 79-80. Nov., 1949.

The open type cabinet can be used effectively by food chains and drug stores for departmentalizing and merchandising carry-home ice cream. Half-gallon and gallon units have proved to be an effective means of getting extra gallonage. The gallon size seems to be too large a unit for the average family unless a low temperature storage cabinet is available. Some manufacturers are experimenting with gallon units which can be broken up into an assortment of quarts and pints in different flavors. During the past year, there has been some development on the west coast in the factory-controlled portion as a means of securing greater dealer cooperation because he will have an assured profit and can figure his costs. "Captive markets", retail chains and food chains that make their own ice cream, are accounting for an increasing volume in many markets.

W. H. Martin

**115. Frozen confection and edible container therefore.** F. L. HARRISON. U. S. Patent 2,489,129. 1 claim. Nov. 22, 1949. Official Gaz. U. S. Pat. Office, **628**, 4: 1169. 1949.

A pastry shell is baked in the shape of a small cylinder with 1 end closed and the other flared outwardly. A plug of ice cream is suspended in the shell, 1 end resting on the bottom and the other flared outwardly and resting on the lip of the pastry shell, but not touching along the straight side.

R. Whitaker

**116. Ice cream cone.** A. A. HEYMAN (assignor to J. Shapiro) U. S. Patent 2,487,136. 6 claims. Nov. 8, 1949. Official Gaz. U. S. Pat. Office, **628**, 2: 393. 1949.

An ice cream cone is described having a paper jacket held in place by ribs molded in the cone.

R. Whitaker

**117. New frozen citrus purees from citrus fruits.** E. A. BEAVENS, Bureau of Agr. and Ind. Chem., Pasadena, Cal. *Ice Cream Rev.*, **33**, 3: 110, 112, 114. Oct., 1949.

Methods of preparation, packaging and freezing have been developed for the preparation of citrus purees which are well adapted for use in the ice cream and other food industries. Sound mature fruit is washed, stemmed, trimmed and crushed. The crushed fruit is reduced to a puree by a mechanically driven screening device with air incorporation kept at a minimum. The yield of puree is 50-60% of the whole fruit and it contains 0.65-0.75% of peel oil. Five parts of puree are mixed with 1 part sugar in a stainless steel tank. The sweetened puree is placed in lacquered or enameled cans of from 1-2.5 gal. capacity. The

cans either are sealed hermetically or closed with slip top covers and the contents frozen rapidly and stored at 0 to -10° F. These purees have been held for more than a year without change in flavor, color and with little or no loss of vitamin C.

Although citrus purees have been used successfully in the preparation of both sherbets and ices, better results were obtained when they were used in sherbets than in water ices. A sherbet mix containing 2.5% fat, 2.5% serum solids, 25% sugar and a suitable stabilizer gave good results with the citrus purees. In the preparation of orange sherbet 15-18 oz. of the 5:1 puree and 1.5 oz. of the 50% citric acid solution/gal. of mix proved satisfactory. When making a lemon sherbet it was found desirable to use only 10-14 oz. of the puree and 0.5 oz. of citric acid/gal. of sherbet mix. W. J. Caulfield

**118. Ice cream freezer.** L. H. KNIBB. U. S. Patent 2,488,668. 8 claims. Nov. 22, 1949. Official Gaz. U. S. Pat. Office, 628, 4: 1048. 1949.

A small freezer for insertion in the freezing compartment of a domestic refrigerator, consists of 2 motor-driven scrapers rotating in a cylindrical vessel. R. Whitaker

**119. Refrigeration cabinet having ice cream can support means.** H. W. CUSTER. U. S. Patent 2,483,264. 1 claim. Sept. 27, 1949. Official Gaz. U. S. Pat. Office, 626, 4: 1124. 1949.

To facilitate dispensing dipped ice cream, this cabinet is so designed that the cans are held at about a 45° angle. R. Whitaker

**120. Clamp for holding spaced ice cream cans.** E. S. CURTIS. U. S. Patent 4,483,038. 6 claims. Sept. 27, 1949. Official Gaz. U. S. Pat. Office, 626, 4: 1069. 1949.

A clamp is described which easily and quickly clamps on the top edge of roundbulk ice cream cans in refrigerated cabinets and holds them rigidly to facilitate scooping of the ice cream. R. Whitaker

Also see abs. no. 75.

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**121. Operational studies of home milk pasteurizers.** R. C. THOMAS, Pub. Health Service, Cin-

cinnati, O. Pub. Health Repts., 64, 45: 1411-1422. Nov. 11, 1949.

The efficiency of 4 home milk pasteurizers was studied by the author. When the units were used according to the manufacturers' directions the phosphatase test of the main body of milk after pasteurization was negative; however, in 3 units positive phosphatase tests sometimes were obtained on the milk swabbed from the inner surface of the container just above the milk level. This was not true in the case of an "in-the-bottle" unit. Two solutions suggested by the author to prevent such positive phosphatase reactions were (a) devise the inner container so that it is completely surrounded by the heating medium and (b) insure that the milk surface and the air above will reach the proper temperature by using higher temperatures throughout the pasteurizing procedure. The double boiler method of heating the milk for 10 min. over vigorously boiling water was found to be satisfactory for heating milk in the home to make it safe for consumption, since no positive phosphatase tests were obtained. However, the temperature of the milk could not be controlled as well and the milk sometimes had a slightly cooked flavor with some precipitation of milk solids. Since the author completed his study, manufacturers of 2 of these units have altered the construction of the tops of the units so that the air temperature above the milk will be raised in hopes of inactivating the phosphatase in the milk swabbed from the inner surface above the milk level. D. D. Deane

Also see abs. no. 80, 86, 109.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**122. Observations on a reflex controlling milk flow in the individual mammary gland of the cow.** E. R. COCHRANE, Tresden, Highworth, Wilts, England. British Vet. J., 105, 8: 320-321. Aug., 1949.

Studies were made on the front teat of a Short-horn heifer which was injured by a cut through the sinus wall. This injury allowed milk to flow without going through the papillary duct. Only a few drops of milk could be removed unless the udder was stimulated, after which the gland milked itself. The flow of milk could be stopped by touching the teat. Over 30 sec. were required before the milk flow resumed. It was concluded that contraction of smooth muscle at the base of the teat closed the duct between the gland and teat sinuses. This contraction probably occurs often on injured teats. B. B. Morgan



## NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

**123. The growth-promoting effect on the rat of summer butter and other fats.** S. LASSEN and E. K. BACON, Van Camp Labs., Terminal Island, Cal. *J. Nutrition*, 39, 1: 83-91. Sept., 1949.

The growth-promoting properties of summer butter, margarine fat, cottonseed oil and olive oil were studied by adding them to a fat-free basal diet at levels of 10% and feeding to growing rats. Body weight changes and body length measurements were the criteria used for comparisons. Summer butter contains no growth factors not contained in margarine fat and cottonseed oils.

R. K. Waugh

**124. Process for improving the digestibility of milk.** O. E. CARMEN. U. S. Patent 2,490,015. 5 claims. Dec. 6, 1949. *Official Gaz. U. S. Pat. Office*, 629, 1: 95. 1949.

Following milking, cows milk is cooled to 40-65° F. and then subjected to agitation by means of a beater which rotates at a speed of 1200-3000 rpm and vibrates at a frequency of from 200-400 osc./sec. for a period of time of 10-30 min. The agitation described partially removes the adsorbed layer from the fat globules, but is not so drastic as to cause rancidity.

R. Whitaker

## PHYSIOLOGY AND ENDOCRINOLOGY

R. R. REECE, SECTION EDITOR

**125. Relation of food intake to growth depressing action of natural and artificial estrogens.** J. MEITES, Michigan State College, East Lansing. *Am. J. Physiol.*, 159: 281-286. Nov., 1949.

The administration of either natural or artificial estrogens can depress growth in rats and mice. Of even greater interest to dairymen is that giving synthetic estrogens to milking goats can depress lactation. How these phenomena are elicited are unknown and the author has shown, by studying growing rats, that growth depression caused by synthetic estrogen administration is mediated chiefly through a resulting lowered food intake. Natural estrogens depressed growth but not by lowered food consumption. Other possible mechanisms of growth depression by administration of natural estrogens are reviewed.

V. Hurst

**126. Influence of desoxycorticosterone acetate on liver and muscle glycogen of adrenalectomized**

**animals.** A. SASS-KORTSÁK, F. C. WANG and F. VERZÁR, Univ. of Basel, Switzerland. *Am. J. Physiol.*, 159, 2: 256-262. Nov., 1949.

Male rats (70-160 g.) were placed on 3 diets: carbohydrate rich, forced fed glucose to starved animals and protein diet. In each group, normal animals were compared on the basis of liver and muscle glycogen to untreated adrenalectomized animals and to adrenalectomized animals receiving desoxycorticosterone acetate (DCA). Animals were sacrificed at intervals varying from 2-28 d. following adrenalectomy. DCA was able to maintain normal muscle and liver glycogen in adrenalectomized animals and its action here helps to substantiate the claim that, given sufficient time to act, DCA, as well as the 11-oxycorticosteroids, can influence carbohydrate metabolism.

V. Hurst

**127. Comparisons between glycogenetic property of desoxycorticosterone, 11 dehydro-17-hydroxycorticosterone (Compound E), and adrenal cortical extract.** F. C. WANG and F. VERZÁR, Univ. of Basel, Switzerland. *Am. J. Physiol.*, 159, 2: 263-268. Nov., 1949.

These hormones were tested for their action on liver and muscle glycogen formation in adrenalectomized rats on a protein diet. Compound E caused a quicker and more powerful action on glycogen formation in a short time period, 6-48 hr., than did DCA, but over longer periods of from 7-15 d., the action of DCA became more evident. The difference in influence on glycogen formation, although still in favor of compound E, assumed a smaller magnitude over the longer time periods.

V. Hurst

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**128. A line of houseflies resistant to methoxychlor.** G. W. BARBER and J. B. SCHMITT, Rutgers Univ., New Brunswick, N. J. *J. Econ. Entomol.*, 42, 5: 484-485. Oct., 1949.

This is a report of further studies of a type published previously and referred to herein. Under the conditions of these laboratory tests, there was 100% kill of 2 laboratory strains of flies with DDT, whereas 1 of these strains similarly succumbed to methoxychlor, but the other did not. A so-called DDT-resistant strain of flies from nature, which had been reared in the laboratory for the tests, showed resistance to both DDT and methoxychlor.

E. H. Fisher

**129. Reaction of certain fly strains to DDT and methoxychlor deposits.** E. J. HANSENS, Rutgers Univ., and A. H. GODDIN, E. I. du Pont de Nemours & Co., Inc. J. Econ Entomol., 42, 5: 843-844. Oct., 1949.

Laboratory insecticidal tests were conducted with flies of 3 laboratory strains and 1 so-called DDT-resistant strain from nature which had been reared to 18th and 19th generations in the laboratory. Plywood panels were treated with either wettable powder slurries or acetone solutions of either DDT or methoxychlor at several deposit rates. When deposits dried, flies were caged over specific areas for 15 min., after which knockdown

and mortality records were made. Kill was judged 1 d. after exposure.

About 2.5 mg. DDT (as wettable powder)/ft.<sup>2</sup> was required to kill 100% of the 3 laboratory strains, and about 15 times more DDT was needed with the resistant strain. Methoxychlor (as wettable powder) was used at 4.7, 7.2, 7.4 and 6.2 mg./ft.<sup>2</sup> to secure 100% kill of the 3 laboratory and the DDT-resistant strains, respectively.

In acetone solution, 144 mg. DDT/ft.<sup>2</sup> killed 100% of the 3 laboratory strains and 25% of the resistant strain. Deposits as great as 576 mg. methoxychlor (in acetone solution)/ft.<sup>2</sup> failed to kill 100% of any fly strain. E. H. Fisher



# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

### BOOK REVIEWS

**130. Cheese.** L. L. VAN SLYKE AND W. V. PRICE. Orange-Judd Publishing Co., Inc., New York, N. Y. 522 pp. \$4.50. 1949.

The many persons who have used and appreciated the first edition of *Cheese* by the same authors especially will be grateful for this thoroughly revised and enlarged volume. Dr. Price has provided an excellent textbook in a field where such has been sorely needed. Although written at the college level, the style is simple and direct. It should be interesting and understandable reading for the practical cheesemaker. Cheddar cheese in its many aspects forms the body of the text but process, cottage and cream cheeses also are well covered. No attempt is made to deal with the so-called "foreign types" of cheese. Especially effective use is made of graphs, charts and line drawings. Extensive references to original literature invite the student to pursue further those aspects of the subject which particularly interest him. Excellent practical discussions on natural cheese in consumer packages and bacteriophage in cheese starters indicate the author's up to date approach. The index covers 12 double-column pages and gives evidence of careful preparation. This volume compares favorably in organization, style and selection of subject matter with our best current college text books.

E. F. Goss

**131. Laboratory Manual for Dairy Bacteriology.** E. M. FOSTER AND W. C. FRAZIER. Burgess Publishing Co., Minneapolis, Minn. 59 pp. Mimeoprint. \$1.75. 1950.

This laboratory manual is intended for use in classes that meet 30-32 laboratory periods for 2 hr. each, with intervals of 2 and 5 days. It is divided into 3 parts, the first of which deals with common microorganisms found in milk. In this part the exercises include the various groups of microorganisms with a study of representative species. The second part takes up the methods used in the control of milk quality. This includes

exercises on the agar plate method and the direct microscopic count for bacteria, methylene blue, resazurin and fermentation tests and the detection of coliforms, along with tests for abnormal milk. The third part is devoted to the microbiology of dairy products, with exercises on starters, evaporated and sweetened condensed milk, butter, cheese, dried milk and ice cream.

Each exercise is followed by a series of related questions. Detail in explanation is kept to a minimum. Explanation of methods and interpretation of results are grouped together in an appendix for easy reference. W. W. Overcast

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**132. Bovine mastitis. Treatment with penicillin and herd practices which aid in its control.** D. F. BREAZEALE, P. L. KELLY, E. BARTLE, A. B. HOERLEIN AND G. S. HARSHFIELD. S. Dakota Agr. Expt. Sta. Bull. 392. 1949.

Treatment of bovine mastitis by penicillin infusion of infected quarters was carried out over a 2-yr. period in the dairy herd of the S. D. Agr. Expt. Station. At the beginning, 55.0% of the herd had infections in 1 or more quarters. At the end of 2 yr., infection had been reduced to 14.6% by herd management practices, treatment and culling.

Penicillin was more effective against streptococci than against staphylococci. Penicillin treatment freed the quarters of *S. agalactiae* in 70.3% of the cases. Treatment was more effective for mild cases of short duration than for well-advanced cases. Penicillin infusions were somewhat less effective for reinfections of *S. agalactiae* than were first treatments. Clinical mastitis occurred more frequently among cows kept in stanchions than among those kept in pen-barns.

J. W. Stull

**133. Characteristics of some strains of streptococci in mastitis.** L. A. BURKEY AND CECILIA R. BUCKNER, Bureau of Dairy Industry, Wash-

ington, 25, D. C. Soc. Am. Bact., Abs. of Papers, p. 51. May, 1949.

At least 5 species of streptococci were found in the Bureau's herds at Beltsville, Md. The percentage of quarters infected with strains of *S. agalactiae* was 10; with *S. dysgalactiae*, 5; *S. uberis*, 26; the viridans group, 8; the enterococcus group, 4; hemolytic staphylococci, 29; pseudomonads, 8; coliforms, 5; unidentified, 6. Strains of streptococci and enterococci were distinguished by their fermentations. Severity of infection, as indicated by leucocytes and percentage of chlorides in milk, was not correlated with a particular organism. Because of mixed infections involving hemolytic staphylococci and streptococci, it is suggested that there may be a natural association of the salicin-positive strain of *S. agalactiae* with the hemolytic staphylococci. However, mixed infections of these organisms were less severe than those with *S. agalactiae* alone. D. P. Glick

**134. The relationship of machine milking to the incidence and severity of mastitis.** E. B. MEIGS, L. A. BURKEY, G. P. SANDERS, M. ROGOSA AND H. T. CONVERSE, U. S. Dept. Agr. Tech. Bull. 992. 51 pp. Aug., 1949.

Twenty-five cows were studied using severe, routine and mild machine milking and hand milking for extended periods in 1 or 2 lactations. Presence and severity of mastitis were defined by leucocyte and *Streptococcus agalactiae* counts, and by chloride determinations on milk samples from separate quarters. Incidence of mastitis was reduced by shortening the time from approximately an average of 14-5 min./cow/milking and reducing the vacuum applied in machine milking from 16-12 in. of Hg. Even less mastitis occurred upon changing from machine to hand milking. High chloride contents were encountered in some milk of normal cows on routine or severe machine milking, even without mastitis occurring. Some mastitis cases were observed in which *S. agalactiae* was absent or in small numbers. Also, milk from some normal cows contained several thousand of this organism/ml.<sup>8</sup> without other evidence of udder injury. Udder injuries caused by severe milking methods decreased milk yields or caused complete loss of secretory function in individual quarters of the udder. R. B. Becker

**135. Field experience with Brucella M vaccine.** B. J. KILLHAM, G. W. REED AND C. F. CLARK. Mich. Agr. Expt. Sta. Quart. Bull., 32, 2: 240-244. Nov., 1949.

Agglutination tests were made on cattle in 81 herds (77 infected with brucellosis) in 2 counties under area test and 36 herds (30 infected) in another county. Some 2,402 animals were vac-

inated with *Brucella M* vaccine and subsequently re-tested. Records on 2,927 unvaccinated cattle were obtained for comparison, based on both initial and 2 retests. Though most herds were found by the initial test to be infected before vaccination, reactors and suspects were reduced about one-half on the subsequent retests, attributable to vaccination. R. B. Becker

**136. Further observations on brucella infections and the role of a selective host-factor affecting variation.** W. BRAUN AND DOROTHY MEAD, Camp Detrick, Frederick, Md. Soc. Am. Bact., Abs. of Papers, p. 86. May, 1949.

Embryonated eggs, which are susceptible to *Brucella*, were inoculated with known mixtures of *B. abortus S* and *R* cells, or *S* and *M* cells. When the cultures were recovered from the eggs, it was observed that the establishment of non-smooth types had been suppressed. On the other hand, studies with chicken sera had indicated that the selective effect is lacking in adult chickens, which are relatively insusceptible to *Brucella* infections. Earlier *in vitro* tests showed that the selective serum factor disappears after infection. This was supported by experiments with guinea pigs which were inoculated with *B. suis* containing a small percentage of a non-smooth type. When animals were sacrificed at different periods after infection, a progressive establishment of the non-smooth type was observed in cultures from spleen and lymph nodes. D. P. Glick

**137. Some considerations on the eradication of bovine tuberculosis.** G. FLUCKIGER, Berne, Switzerland. British Vet. J., 105, 11: 401-414. Nov., 1949.

The first steps for a systematic program for combating this disease in Switzerland was started in 1934. By 1948, 1/5th of all the national herd was under official control. Extensive areas are now practically free from bovine tuberculosis. Some of the problems encountered were economic rather than scientific. The various methods of combating the disease in Switzerland are discussed. It was pointed out that the eradication of the disease is easy where the incidence is low and very difficult where it is high. At the present time approximately 20% of the cattle in Switzerland show tuberculosis lesions.

B. B. Morgan

Also see abs. no. 162, 163, 211.

## BUTTER

O. F. HUNZIKER, SECTION EDITOR

**138. Character of Ontario butter.** F. W. HAMILTON, A. G. LEGGATT AND W. H. SPROULE. Can. Dairy Ice Cream J., 28, 12: 44-57. Dec., 1949.

A study was made of the character of Ontario butter based upon analyses of samples submitted over the past 4 yr. to the Dairy Dept. of the Ontario Agricultural College. Of the cream received during the summer season, 74.8% had an acidity ranging from 0.41%–0.6%. After neutralization most of the cream had an acidity of 0.15%–0.20%. The pH of the butter serum recognized for best keeping quality is pH 6.6–7.2, 56.8% of Ontario butter in 1946 fell within this range. By 1949, 61.9% of the total churnings fell within the desired pH range. The results indicate a lack of uniformity in salt control. Moisture content appears to be well controlled. A large proportion of the butter scoring 1st grade into storage dropped to 2nd grade during the storage period of 10 mo. Flavor defects encountered most frequently were stale, unclean neutralizer and metallic. Improvement could be made on yeast and mold content on some of the butter. H. Pynson

**139. Aluminum foil vs. parchment for wrapping print butter.** A. H. WHITE. Can. Dairy Ice Cream J., 28, 9: 27–29, 80. Sept., 1919.

Aluminum foil wraps were compared with parchment for packaging print butter for storage at 10° F. and 28°–30° F. for periods ranging from 15–37 wk. Butter put up in foil wraps prevented flavor deterioration at the surface of the prints and the butter maintained first grade quality. The surfaces of the butter wrapped in parchment deteriorated enough in flavor to put the butter in second grade. At 10° F., loss of weight was slightly less and more uniform color was maintained with foil wrap than with parchment. At 28–30° F. with high humidity, butter in both wraps maintained good color, and the loss in weight was less for the parchment-wrapped prints than for prints in foil. The results indicate the possibility of storing high quality print butter in aluminum foil at cold storage temperatures for periods up to 37 wk. without loss of grade. H. Pynson

**140. Use of milk fat fractions in baked products.** LURA M. MORSE AND E. L. JACK, Univ. of Cal., Davis. Food Research, 14, 4: 320–324 July–Aug., 1949.

Studies were made, of the use in baked products, of 2 fractions of milk fat, 1 precipitated from solvent at –20° C., the other at –53° C. The higher solidifying fraction was more suitable as a shortening in cake than the lower solidifying fraction. In pastries the 2 fractions reversed their roles and the –20° fraction was not suitable for

the ice water method, whereas the –53° fraction could be used successfully if chilled to 7.8° C. F. J. Doan

## CHEESE

A. C. DAHLBERG, SECTION EDITOR

**141. Milk pasteurization for cheese making.** N. S. GOLDING AND I. ERICKSEN. Can. Dairy Ice Cream J., 28, 9: 68–74. Sept., 1949.

Four organisms which had been found to survive pasteurization and grow in milk were added to the cheesemilk before pasteurization. With the exception of 1 culture, these organisms greatly increased the count of the pasteurized milk. During the cheese making procedure, all of the organisms except one showed a rapid multiplication in the cheesemilk. The addition of the cultures to the cheesemilk did not change significantly the gas production in the cheese. Two of the cultures slightly decreased the quality of the cheese below that of the control cheese at low ripening temperatures. It is questionable whether the slight differences in quality of the cheese could be attributed to the cultures used. H. Pynson

**142. Effect of added micrococci on flavor development in Cheddar cheese from pasteurized milk.** J. A. ALFORD AND W. C. FRAZIER, Univ. of Wisconsin, Madison. Soc. Am. Bact., Abs. of Papers, p. 53. May, 1949.

Selected strains of micrococci were isolated from young, raw milk cheese. A 1–3% inoculum of *Micrococcus* sp. was added with lactic starter to pasteurized milk cheeses. The cheeses were ground after 2–4 wk. to hasten development of flavor. Two strains of *Micrococcus* produced an enhanced flavor development, compared with control lots. Both strains developed rapidly during manufacture and pressing and reached maximum count by the 2nd d. One strain decreased rapidly after the 3rd–4th d. and disappeared almost completely in 2–4 wk. The other strain died very slowly. Continued viability may not be essential to flavor development. There was no relation to increases in water soluble nitrogen and total volatile acidity. D. P. Glick

**143. The use of a semi-automatic moisture tester for cheese and cheese products.** F. V. KOSKOWSKY, A. C. DAHLBERG AND B. L. HERRINGTON, Cornell Univ., Ithaca, N. Y. Food Tech., 3, 9: 320–322. 1949.

The operation of the Brabender semi-automatic moisture tester is described. Only 1 outside weighing is required with this moisture tester, the final weighing being conducted while the pans

are in the oven. The temperature of the oven is controlled by an electrical contact thermostat adjustable from 45°-160° C.

Rates of drying curves obtained on American cheddar cheese, mild process cheese food and sharp process cheddar cheese are shown. For American cheddar cheese, a temperature of 135° C. for 45 min. gave the most accurate results. Age and moisture level of the cheese had some effect upon the final moisture percentage, but the variations were not very significant. For mild process cheese food and aged process cheddar, a temperature of 140° C. for 1 hr. was required. A full oven of 10 pans did not give the same results on the same cheese as an oven that contained only 2 pans.

E. R. Garrison

#### 144. Microbiological determination of free amino acids in cheese during the curing period.

D. G. REIHARD AND J. C. GARFY, Pennsylvania State College, State College. Soc. Am. Bact., Abs. of Papers, p. 52. May, 1949.

To obtain complete extraction of free amino acids, cheese was dried *in vacuo*, fat removed by ether extraction in the Soxhlet apparatus and the lipid-free cheese then was subjected to 4 successive extractions with boiling water. Each water extraction was followed by centrifugation. The 4 samples of supernatant fluid were combined for assay. The free amino acid content of American cheese did not change during manufacture but significant increases developed thereafter. Cheese after pressing was used as reference. After 2 wk., leucine and isoleucine increased 2-fold and glutamic acid increased 5-fold. At 12 wk. there was a 2-fold increase in threonine, methionine and lysine; 4-fold increase in leucine and isoleucine; 3-fold increase of valine and 7-fold increase of glutamic acid. Free tyrosine, tryptophane and histidine were found but without significant increases in content. Free amino acids of cheese made from raw milk increased more rapidly than in cheese made from pasteurized milk. Similar data on Trappist cheese were found.

D. P. Glick

#### 145. Enriched cottage cheese. Anonymous. Milk Dealer, 38, 11: 45, 106. Aug., 1949.

A number of milk dealers are now marketing an enriched cottage cheese which contains not only vitamin D, to make a rich source of Ca and P available to the consumer, but also vitamin A to increase its nutritional value. Fortification now opens new sales possibilities for this product.

C. J. Babcock

Also see abs. no. 130, 151, 152, 153, 154, 156, 157.

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

#### 146. The body of cultured cream. E. S. GUTHRIE. Can. Dairy Ice Cream J., 28, 9: 34-35. Sept., 1949.

The firmness and the viscosity of cultured cream can be controlled largely by proper homogenization and pasteurization of the original cream. A temperature of 165° F. for 30 min. and homogenization at 3000 lb./in.<sup>2</sup> pressure or rehomogenization at the same pressure and temperature gives a firm dry desirable body. Firmness of the cultured cream is evaluated with a plummet described by Hilker (J. Dairy Sci., Mar., 1947).

H. Pycnson

#### 147. Consumer reaction to bottled fresh concentrated milk. G. M. TROUT AND G. G. QUACKENBUSH, Mich. Agr. Expt. Sta. Sou. Dairy Prod. J., 46, 5: 68-69, 74-75. Nov., 1949

Consumer reaction on bottled fresh concentrated milk in the ration of 2: 1 and 3: 1, homogenized and pasteurized was obtained.

In spite of low forewarming temperatures, cooked flavor predominated in the reconstituted product. Homogenization pressures of 2,000-2,500 lb. were adequate to maintain satisfactory homogeneity. The type of water used affected the flavor of the reconstituted milk, but this was not believed to be a serious factor. The noticeable cooked flavor was not objectionable to the majority of the consumers. Most of the consumers were interested in saving refrigerator space by using the concentrated product, but not interested in buying it unless they could save 2 or 3¢/qt. of milk equivalent. The majority of customers believed that the product had commercial possibilities.

F. W. Bennett

Also see abs. no. 140, 150, 215, 218.

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

#### 148. An analysis of several stains used and proposed for the direct microscopic enumeration of the bacteria in milk. M. BRANDSTEIN AND J. O. MUNDT, Univ. of Tenn., Knoxville. Food Tech., 3, 10: 324-326. 1949.

Direct microscopic bacterial counts were made on the dried smears prepared from 61 producer milk samples and stained by 6 staining procedures. The counts obtained by each staining method were compared statistically to determine maximum numbers and deviations. Twelve characteristics desired in a good stain were listed and the 6

stains were evaluated thereby. According to this method of evaluation North's stain received a total of 65 out of a possible 72 points in scoring. The points scored by the other stains used in this study were as follows: Breed 45, Broadhurst-Paley 42, Mandel 40, Watrous-Doan 37 and Gray 22.

E. R. Garrison

**149. An improved microscopic method of examining fatty foods.** T. H. LORD AND MARGARET M. SMALL, Kansas State College, Manhattan. Food Research, 14, 3: 241-242. May-June, 1949.

The use of a surface active agent (Tide) in the preparation of a fatty food (oleomargarine) for microscopic examination to observe number and type of microorganisms was found to give greatly superior results to those obtained with Fay's method originally proposed for butter. The suggested technique results in vastly greater homogeneity in the appearance of the slide and in the prevention of clumping of bacterial cells.

F. J. Doan

**150. Comparison of methods of reconstituting milk powder for the plate count with an analysis of variance.** F. J. CONI, AND U. S. ASHWORTH, State College of Washington, Pullman. Food Research, 14, 2: 165-176. Mar-Apr., 1949.

In this study, wherein spray powders only were used and the results subjected to statistical analysis, reconstitution with water at 45, 50 or 55° C. gave much higher bacteria counts (plate) than when water at room temperature was employed. The use of mechanical shaking, with and without beads, for periods of 2, 5 and 15 min., when the powders were reconstituted with water at room temperature, resulted in no significant increase in count.

Alkaline water had no measurable effect in increasing the dispersion of bacterial cells as far as could be judged from the counts, but LiOH (N/10) actually lowered the number of colonies developing on the plates.

Water at 50° C. and quarter-strength Ringer's solution (50° C.) as the reconstituting medium gave closely agreeing results, while solutions of dilute Na citrate and Na<sub>2</sub>HPO<sub>4</sub> appeared to interfere with the so-called "heat activation" of the cells.

F. J. Doan

**151. Inhibition of lactic organisms by cheese starter cultures.** L. E. BARIBO AND E. M. FOSTER, Univ. of Wisconsin, Madison. Soc. Am. Bact., Abs. of Papers, p. 52. May, 1949.

*Lactobacillus casei* was inhibited when grown in milk with *Streptococcus lactis*. Inhibition was not due to acid production nor to competition for food. Growth of *L. casei* was inhibited when

heat-killed cultures of lactic streptococci were added. After 4-5 d. the effect was overcome. Three commercial cheese starters inhibited *L. casei*. Inhibition was demonstrated in whey samples from a Cheddar cheese vat and in curd after removal from the press. The inhibitory factor was heat-stable at acid reaction but was labile in the presence of alkali.

D. P. Glick

**152. Penicillin in milk. A hazard to starters, buttermilk and cottage cheese manufacture.** W. A. KRIENKE, Florida Agr. Expt. Sta., Gainesville. Milk Dealer, 39, 2: 126-129. Nov., 1949; also Southern Dairy Prod. J., 46, 6: 32, 38. Dec., 1949.

Milk containing as little as 0.10 I.U. of penicillin/ml. of milk developed 0.46% titratable acidity, as compared with the non-penicillin control of 0.75% titratable acidity, when incubated at 68-70° F for 16 hr; after 24 hr. the values were 0.59 and 0.80%, respectively. When 1.0, 0.50 and 0.25 I.U.'s of penicillin were present in the cultured milk, the acidity developed to 0.26, 0.25 and 0.27%, respectively, during a 16-hr. incubation period.

Regardless of the heat treatment given the milk containing 1 I.U. of penicillin/ml. of milk, 143° F. for 30 min., 190° F. for 60 min., 10 lb. steam pressure in an autoclave for 15 min., or 15 lb. steam pressure for 15 min. followed by temperature adjustment to 70° F. and subsequent inoculation with 1% of culture, the titratable acidity did not exceed 0.24% within 18 hr. when incubated at 68-70° F.

Penicillin in milk from cows treated for mastitis is a hazard to starters, buttermilk and cottage cheese.

C. J. Babcock

**153. Penicillin and cheese making.** E. G. HOOD AND H. KATZNELSON. Can. Dairy Ice Cream J., 28, 10: 27. Oct., 1949.

Penicillin in cheese factory milk and milk used for starter making may present a major problem as the use of penicillin in the treatment of mastitis becomes more extensive. Penicillin in starter milk is not completely inactivated at temperatures as high as 185°-190° F. Therefore, care should be exercised in milk selection and only milk from healthy animals should be used.

H. Pyenson

**154. Penicillin in relation to lactic acid streptococci in starter cultures used in Cheddar cheese-making.** H. KATZNELSON AND E. G. HOOD, Dept. of Agr., Science Service, Ottawa, Canada. Soc. Am. Bact., Abs. of Papers, p. 53. May, 1949.

Penicillin, 50-100 units/100 ml. or pasteurized milk, completely inhibited acid production by a mixed or a single strain starter culture. Partial



inhibition was obtained with 0.5–5.0 units. Neither pasteurization nor cysteine inactivated penicillin. Penicillinase, 0.02 mg./100 ml. milk, overcame the effect of 5–10 units of penicillin and permitted acid production in the presence of 100 units. Of 44 strains of streptococci tested in whey broth and in skim milk, all were more sensitive in whey broth, most being inhibited completely at a dilution of 1:16–1:32 million. Some were inhibited at 1:128 and 1:256 million. In skim milk, all strains were inhibited by dilutions ranging from 1:4–1:16 million and most by 1:8 million. Using graded amounts of penicillin in milk, increased resistance of starter cultures could be developed. Four out of 6 commercial starters gave normal coagulation of 100 ml. milk in 24 hr. at 18° C. in the presence of 13 units of penicillin. D. P. Glick

**155. The effect of quaternary ammonium compounds on lactic starter cultures.** F. W. BARBER, H. P. HODES AND ANNA M. DUNNE, National Dairy Research Labs., Inc., Oakdale, N. Y. Soc. Am. Bact., Abs. of Papers, p. 56. May, 1949.

Milks containing various concentrations of quaternary ammonium compounds and cleaner-antibacterizer compounds were inoculated with a 3% inoculum of an 18-hr. culture of *Streptococcus lactis*. In concentrations up to 10 p.p.m. of active ingredient, development was normal after 18 hr. incubation. Acid production decreased and chaining of cells increased at concentrations of 25–50 p.p.m. Concentrations of 100 p.p.m. inhibited growth and acid production. D. P. Glick

**156. Factors affecting the quantitative measurement of *Streptococcus lactis* bacteriophage.** W. B. CHERRY AND D. W. WATSON, Univ. of Wisconsin, Madison. Soc. Am. Bact., Abs. of Papers, p. 22. May, 1949.

Two methods of measuring *Streptococcus lactis* virus, plaque count and lytic activity, have errors not to exceed 13%. Both are subject to variations unless pH, age, activity of cell suspensions and composition of medium are controlled rigidly. Initial pH of medium used is 7.0; if pH drops below 5.0, lysis does not occur. Buffering with phosphates prevents virus adsorption. Best results have been obtained using cells 3–5 hr. old resuspended in normal saline. By such standardization, 1-step growth curves can be used to define virus growth characteristics. D. P. Glick

**157. Proliferation of bacteriophage on *Streptococcus lactis*.** F. E. NELSON AND C. E. PARMELEE, Iowa Agr. Expt. Sta., Ames. Soc. Am. Bact., Abs. of Papers, p. 22. May, 1949.

Changes in bacteriophage and *S. lactis* popula-

tions in milk incubated between 21 and 39° C. were followed for such periods of time as to obtain mass lysis of susceptible cells. Bacteriophage particles were counted by plaque procedures in which variations in technique had been adjusted to give maximum counts of the 5 different bacteriophage strains used. Changes in pH were followed. Bacteriophage proliferates at a greater relative rate than that of sensitive *S. lactis* at temperatures favorable to bacteriophage. Optimum temperature for multiplication of both was 32° C. One bacteriophage failed to multiply at 35, another at 37 and a third at 38.5° C., although the homologous bacterium multiplied in each case.

At favorable temperatures, each bacteriophage proliferation curve showed a lag phase, log growth phase and a maximum stationary phase. The maximum level was approximately 10<sup>9</sup> bacteriophage particles/ml. of culture in most cases, irrespective of strain, host organism and some variation in temperature. Acid production of bacteriophage-infected cultures stopped by the time mass lysis occurred, or earlier. Different cultures gave counts from 0–10,000 secondary organisms/ml. at the time of mass lysis, variations depending upon the combination of bacteriophage and bacterium used. D. P. Glick

**158. Antigenic interrelationships among certain bacteria of the lactobacillus group.** F. J. ORLAND, Univ. of Chicago. Soc. Am. Bact., Abs. of Papers, p. 76. May, 1949.

Some 200 strains of lactobacilli were collected or isolated and observed for antigenic characteristics by means of agglutination tests and by agglutinin absorption. The antigen *F* was easily detectable in certain strains of *L. casei*, *L. delbrueckii*, *L. bulgaricus*, *L. helveticus* and *L. acidophilus*, and in a few strains from human saliva of individuals with carious teeth. The *F* antigen was associated with the ability to ferment both rhamnose and sorbose. Antigens *G* and *H* were found in a strain of *L. plantarum* and of *L. casei*, respectively, neither of which contained *F* antigen nor fermented rhamnose or sorbose. Agglutinin absorption indicates that the antigenic components are separate entities. D. P. Glick

**159. Studies on cellulolytic bacteria in the bovine rumen.** R. E. HUNGATE, Washington State College, Pullman. Soc. Am. Bact., Abs. of Papers, p. 61. May, 1949.

Freshly isolated strains of rods and colorless and yellow cocci were studied. Subculturing in liquid media is conducive to cellulolysis. Rumen fluid is essential to growth of the rods and the colorless cocci and cannot be replaced by any substrates tested. The nutritive value of rumen fluid is due

to associated microorganisms. The rods ferment cellulose, cellobiose, glucose, starch, dextrin, maltose and trehalose. The colorless cocci ferment only cellulose and cellobiose. The rods produce no copper-reducing materials in old cultures containing excess cellulose and the colorless cocci produce only small amounts. The yellow cocci produce cellobiose and small amounts of glucose. Yellow cocci fermentation products are  $H_2$ ,  $CO_2$ , ethyl alcohol, acetic acid and lactic acid, lactic acid being the most important quantitatively. The colorless cocci produce the same substances and formic acid. Only acetic and succinic acids have been identified as fermentation products of the rods.

D. P. Glick

**160. Salt tolerance of the coliform bacteria.** O. FODA AND R. VAUGHN, Univ. of Cal., Berkeley. Soc. Am. Bact., Abs. of Papers, p. 54. May, 1949.

Salt-tolerant coliforms were isolated by enriching cucumber and olive brines in nutrient glucose broth containing 10% salt. An unusual type of *Aerobacter aerogenes* was identified. The isolates grew in the presence of more than 13% NaCl after adaptation. These will tolerate 10.5 to 11% salt without adaptation and acquired tolerance is lost only after 8 or more transfers in a salt-free environment. Typical coliforms can tolerate about 9.5% salt only after a 6-mo. period of adaptation and this is lost after 1 transfer in a NaCl-free medium. Without adaptation, the tolerance of typical coliforms is 6.5–7.5% salt.

D. P. Glick

**161. The oxidation of amino acids by brucellae.** P. GERHARDT AND J. B. WILSON, Univ. of Wisconsin, Madison. Soc. Am. Bact., Abs. of Papers, p. 40. May, 1949.

Strain 19 was used with Warburg manometric techniques. Of 25 compounds, only L-glutamic acid, L-asparagine and DL-alpha-alanine were oxidized at an appreciable rate; this was accompanied by deamination and decarboxylation. L-glutamic acid was oxidized at a rate greater than glucose or any other compound tested; L-asparagine and DL-alanine were oxidized at lesser rates. The system was specific for L-isomers in the case of glutamic acid and asparagine. Both L- and D-forms of alanine were utilized at approximately the same rate. Oxidative specificity of the organism for glutamic acid, asparagine or alanine may be correlated with utilization of amino acids as the sole nitrogen source in chemically defined media.

D. P. Glick

**162. Dissociated growth phases of brucella and their properties.** I. F. HUDDLESON, Michigan State College, East Lansing. Soc. Am. Bact., Abs. of Papers, p. 11. May, 1949.

A small colony phase (0.1 mm or less) has been obtained from *Brucella abortus* which has the characteristics of the S Phase, but always dissociates into a large colony I phase. Five mucoid phases have been obtained from *Br. suis*; each has a different dissociation pattern. Three different mucoid phases and an SL phase have been obtained from *Br. melitensis*. All mucoid phases but one fail to produce progressive disease in experimental animals. Two suis mucoid phases are highly immunogenic.

D. P. Glick

**163. Sensitivity changes of *Actinomyces bovis* to penicillin and streptomycin.** A. BOAND AND M. NOVAK, Univ. of Illinois Medical School, Chicago. Soc. Am. Bact., Abs. of Papers, p. 76. May, 1949.

Six strains of *Actinomyces bovis*, 4 from human cases and 2 of bovine origin, were tested to determine whether *A. bovis* would become antibiotic-resistant during prolonged contact *in vitro*. The desired concentration of antibiotic was incorporated in thioglycollate medium. Penicillin sensitivities differed somewhat but all were inhibited by 0.5 unit/ml. Streptomycin inhibited the 6 strains at 30 units/ml.; slight growth occurred at 20 units. Only slight tolerance to penicillin was developed by 4 of the 6 strains during the 1st 16 transfers, this tolerance was not increased after 32 transfers over 3 mo. All strains rapidly developed a high degree of resistance to streptomycin, growing in 5,000 units/ml. on the 10th transfer. Five strains retained full resistance after 54 transfers in streptomycin-free medium; 1 returned to its original sensitivity after 45 transfers. Development of resistance and reversion occurred in a step-wise manner, suggesting the possibility of genetic changes.

D. P. Glick

**164. Germination of anaerobic spores induced by sublethal heating.** H. REYNOLDS AND H. LICHTENSTEIN, Bur. of Human Nutrition and Home Economics, U.S.D.A., Washington, D. C. Soc. Am. Bact., Abs. of Papers, p. 9. May, 1949.

Portions of a suspension of Cameron's putrefactive anaerobe (P.A. 3679), consisting of spores and vegetative cells, were sealed in Pyrex thermal death time tubes, heated at 104° C. and samples removed at intervals for making survivor counts. Instead of a sharp initial drop in numbers, the apparent viable counts increased from  $2.5 \times 10^6$  to  $16.5 \times 10^6$ /ml. during the first 16 min. of heating. Continued heating resulted in lower counts. In subsequent tests, apparent viable counts were increased from 3–10 fold by heating at 100° C. for 20 min.

It is suggested that heat treatments required for activation may be directly related to natural heat resistance. A strain of *Clostridium botulinum* less than half as resistant as P.A. 3679 at 120° C. was activated when heated for 20 min. at 70, 80 or 90° C. but counts were decreased by heating at 100° C. Modification of the counting medium did not substitute for heat activation.

D. P. Glick

**165. The simplification and standardization of microbiological assays in the control laboratory.**

BERVI, F. CAPPS AND N. L. HOBBS, R. P. Scherer Corp., Gelatin Products Div., Detroit, Mich. Soc. Am. Bact., Abs. of Papers, p. 8. May, 1949.

Microbiological vitamin assay methods have been simplified by adopting a basic dilution pattern common to all assays. Working dilution, basal medium and test organism are the only variables. Commercial, dehydrated media are used to prepare basal media. Aluminum caps are used to replace cotton plugs. Sterilization time has been reduced to 3 min. at 121–123° C. This reduces caramelization so that turbidimetric evaluation is more satisfactory. Turbidimetry following 18–20 hr. incubation is preferred to the 72-hr. titrimetric method.

D. P. Glick

**166. Accuracy and sensitivity of fermentation tests.** H. D. VERA, Baltimore Biological Lab., Baltimore, Md. Soc. Am. Bact., Abs. of Papers, p. 6. May, 1949.

Many late and variable fermentation reactions may be attributed to the presence of fermentable carbohydrates in the peptones used in the media. Fifteen cultures were used to test 300 samples of 23 peptones. Gelatin peptones were negative. Casein peptones (2%) were positive, as were all other peptones examined. Incorporation of meat extract into otherwise carbohydrate-free substrate gave 33% positive results. In addition to the use of substrates tested for freedom from fermentable materials, an indicator changing color at about pH 7.0 is recommended.

D. P. Glick

**167. Methods for determining the ability of yeasts to metabolize nitrate and nitrite.** L. J. WICKERHAM, K. A. BURTON AND R. J. GILL, Northern Regional Research Lab., Peoria. Soc. Am. Bact., Abs. of Papers, p. 7. May, 1949.

Errors in nitrate reduction and assimilation tests have led to conflicting statements concerning yeast taxonomy. False negatives may be obtained with yeasts which metabolize nitrite as rapidly as it is produced from nitrate. This may be corrected by inoculating 4 tubes of nitrate medium and testing after 2, 4, 8 and 12 d. One or more of the cultures will give a positive nitrite test.

The assimilation test, properly used, gives fewer doubtful reactions. Common errors and means of their elimination are: (1) Impurities which serve as sources of nitrogen for growth. Use liquid medium containing no unwashed agar. (2) Addition of vitamin carriers, such as yeast extract, may add sufficient nitrogen to give a false positive test. The use of pure vitamins eliminates this source of error. (3) Incubation period may be too short. Some yeasts assimilate nitrogen strongly only after a period of adaptation.

Some yeasts assimilate nitrite but are without action on nitrate. Thus *Debaromyces* may have a role in the spoilage of brined or cured meats.

D. P. Glick

**168. Bacteria—friend and foe.** N. E. LAZARUS, Lazarus Laboratories, Inc., Buffalo, N. Y. Milk Dealer, 39, 3: 53–60. Dec., 1949.

This is a review of the early history of bacteriology followed by a discussion of bacteria and methods of studying their characteristics and actions.

C. J. Babcock

Also see abs. no. 133, 141, 142, 144, 224, 240, 245, 246.

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**169. Variations in the compositional quality of milk.** G. A. RICHARDSON. Can. Dairy Ice Cream J., 28, 10: 36–37. Oct., 1949.

The relationship between the fat and non-fat solids of milk are entirely irrational and do not conform to the chemical analyses of thousands of samples of genuine milks. The wide variation in the standards recognizes the great variation in the solids of natural milk. In 1 study in New York involving 200,000 samples of genuine milk, average values for fat and non-fat solids were plotted and the conclusions were drawn that no single simple equation could be established to express the relationship over the entire range of fat values. In a study in Boston, a straight-line relationship between the fat and solids-not-fat was established. The equation representing this relationship is:  $S.N.F.\% = 0.4F + 7.07$ . Most equations are not accurate for all milks. A table is given showing the composition of milk in relation to fat content that the author feels is fairly accurate.

H. Pyenson

**170. Method of purifying lactalbumin.** W. E. TRUCE. (Assignor to Swift and Co.) U. S. Patent 2,494,148. 6 claims. Jan. 10, 1950. Official Gaz. U. S. Pat. Office, 630, 2: 493. 1950.

To facilitate the removal of lactose and minerals from lactalbumin, the usual gel formation found when this material is pptd. from whey, the moist albumin curds separated by heat from whey are heated for about 5 min. at about 100° C. The process is discontinued before the lactalbumin is hydrolyzed. After the destabilizing step, the product is washed, dispersed and dried.

R. Whitaker

**171. Hydrolysis of casein.** J. A. REYNIERS. (Assignor to Amino Acids, Inc.) U. S. Patent 2,493,777. 2 claims. Jan. 10, 1950. Official Gaz. U. S. Pat. Office, 630, 2: 398. 1950.

A highly nutritional water soluble hydrolysate is made by ppting. casein from fresh skim milk in a fine flocculent condition by an acid at a temperature of 20° C. or below, then dissolving the casein in an acid and heating the solution to hydrolyze the protein to a point between peptones and amino acids and finally neutralizing, removing the resultant salt and drying to a powder.

R. Whitaker

**172. Vitamin A in milk. Microestimation with activated 1,3-dichloro-2-propanol.** A. E. SOBEL AND A. A. ROSENBERG, Jewish Hospital of Brooklyn, Brooklyn, N. Y. Anal. Chem., 21, 12: 1540-1543. Dec., 1949.

Activated 1,3-dichloro-2-propanol was used as a colorimetric reagent in the determination of vitamin A and carotene in samples of human milk as small as 0.25-1.0 ml. Application of this reaction in place of the usual Carr-Price reaction has several advantages. The color was stable for 8 min., the reagent was non-corrosive, the reagent was not affected by extreme humidity and the use of specially purified chloroform and petroleum ether was not required. Results were close to those obtained when vitamin A was determined by antimony trichloride and carotene by light absorption at 440 mu.

B. H. Webb

**173. Butterfat in ice cream.** A. H. WHITE. Can. Dairy Ice Cream J., 28, 11: 45-49. Nov., 1949.

The perchloric-acetic acid butterfat test for ice cream appeared to be of sufficient merit to warrant further investigation on various types of ice cream. This author has verified some of the results obtained by the original authors of the test. The new test is rapid, gives clear fat columns and readings that are in close agreement with those by the Mojonnier method for most types of ice creams. The straining of fruits, nuts, etc. from ice cream gives more accurate results. Some chocolate ice creams give low inaccurate results due to a formation of a plug

of cocoa in the neck of the bottle, causing a mechanical obstruction to the rising of the fat. The test cannot be applied to ice cream or mixes which contain even a few drops of formalin as a preservative. The cost per test by the perchloric acid method is more than for the H<sub>2</sub>SO<sub>4</sub> notification but the saving in time compensates somewhat for the increased cost.

H. Pyenson

Also see abs. no. 143.

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**174. Studies on the H.T.S.T. pasteurizer.** W. K. JORDAN AND R. F. HOLLAND. Can. Dairy Ice Cream J., 28, 9: 33. Sept., 1949.

Tests have been conducted to determine the effects of the size of pipe used in the holding tube, the velocity of flow through it, the slope of the tube, the percentage of air in the tube and the suction pressure at the pump inlet. The results indicate that increasing the vacuum at the pump inlet decreases the discharge from the pump and with a given initial vacuum pressure, the discharge diminishes as the amount of air leaking into the pump inlet increases. The actual velocity of the liquid is greater when both air and liquid are flowing through the holding tube than when the same quantity of liquid is flowing through the tube with no air present. The flow in the tube is not affected by changes in the slope when the tube is filled completely with liquid. When air is present, the velocity at which the air bubbles move through the tube increases with the slope. In the region of turbulent flow, the holding tube efficiency increases slightly as the average velocity of the flow increases. The presence of air in the holding tube results in a decrease in the efficiency. The efficiency is reduced by about 3-5% with 5% of air in the tube.

H. Pyenson

**175. Milk cooler having automatic control means.** C. H. KAUFER AND H. D. WHITE. (Assignors to Revco, Inc.) U. S. Patent 2,494,512. 12 claims. Jan. 10, 1950. Official Gaz. U. S. Pat. Office, 630, 2: 588. 1950.

A cabinet contains water maintained at a definite level by means of a standpipe and refrigerated by means of a coil in which a cooled compressed gas is allowed to expand. A pump circulates the water around the coils.

R. Whitaker

**176. Food and cream freezer.** D. L. CALMES. U. S. Patent 2,491,952. 1 claim. Dec., 20, 1949. Official Gaz. U. S. Pat. Office, 629, 3: 783. 1949.

A vertical cylindrical ice cream and other food

freezer has a central shaft which rotates a series of short blades against the outer wall. Between the shaft and each blade, a flat inclined plate causes agitation. The vessel is enclosed in a larger chamber in which a refrigerant is placed.

R. Whitaker

177. **Air vent.** F. DURAN. U. S. Patent 2,493,861. 3 claims. Jan. 10, 1950. Official Gaz. U. S. Pat. Office, 630, 2: 419. 1950.

To relieve any pressure which may develop in a covered milk can, the cover is provided with a protected vent which is located entirely within the cover.

R. Whitaker

178. **Glass sanitary piping in dairy plants.** E. THOM. Milk Dealer, 39, 1: 42-43, 134-138. Oct., 1949.

Commercial use of pyrex heat resilient glass piping is expanding rapidly and proving a time and labor saver in the dairy plant. Since 1941 some 20 installations have been made in dairy plants. These installations have been primarily for the movement of raw milk, since those lines are generally the longest, and, therefore, the most difficult to clean. Complete recommendations are given for installing, cleaning and sterilizing glass pipe lines.

C. J. Babcock

179. **Proper lighting in the dairy plant.** H. L. MITTEN, JR., Ohio State Univ., Columbus. Milk Dealer, 39, 2: 48-49. Nov., 1949, *ibid.*, 39, 3: 50-51, 74-76. Dec., 1949.

The benefits of proper illumination are better workmanship, increased production, improved housekeeping, less breakage, better utilization of floor space and fewer accidents. A table is presented showing the recommended minimum standards of illumination for the fluid milk industry. The distribution of light from windows is poor, since the intensity is great near windows and inadequate near the opposite wall. A chart is presented showing that when the illumination 4 ft. from a window is over 20 ft. candles it is approximately 2 ft. candles 24 ft. from the window. Artificial illumination, therefore, provides the only dependable means of controlling the intensity, quality and distribution of light.

Proper illumination is more than the correct intensity, for it must also be of good quality. Lighting quality is made up of intensity, absence of direct and reflected glare, absence of harsh shadows, uniform distribution, properly illuminated surroundings and color of light. Tables are presented showing the lumen output of 110-120 volt Tungsten gas-filled and fluorescent lamps.

C. J. Babcock

180. **Dairy waste prevention and disposal.** H. A. TREBLER AND H. G. HARDING. Can Dairy Ice Cream J., 28, 9: 44, 84. Sept., 1949.

Where waste disposal is a problem, the following steps should be taken: (1) utilize all by-products, such as whey and buttermilk, or dispose of them by some method other than by dumping them down the drains; (2) reduce waste to a minimum by improved preventive maintenance by an educational campaign, by collecting all drips and rinses, by installing automatic pump stops, etc.; (3) install a separate septic tank for toilets; (4) equip evaporatory equipment with entrainment separators in such a way as to give absolute minimum boil-over and entrainment loss; (5) provide an automatic sampler of dairy plant waste and post results of analysis so that plant employees can see results; (6) reduce the volume of waste to a minimum by the installation of a hot water system and automatic shut-off nozzles on the hoses; and (7) install an aerated flow equalizing tank for the floor waste.

H. Pyenson

181. **Treatment of controlled dairy waste in the milk plant.** E. F. GLONA, Univ. of Tex. (in cooperation with Prewitt Creamery, Austin, Tex.) Sou. Dairy Prod. J., 46, 4: 84-89. Oct., 1949.

A satisfactory high-capacity experimental trickling filter with centrifugal recirculation pumps is described. The filter flies which were present were controlled by the spraying of a commercial DDT solution. A thread-like segmented red earth worm would make its appearance whenever the plant was operating under septic conditions. The worm is known as the Dero worm. The bulk of the worms disappeared after washing the drain pan, detention tank and recirculation tank with tap water which had some residual chlorine in it. The remaining worms in the plant after tap water treatment were not very active.

F. W. Bennett

182. **Dielectric defrosting.** E. ROSS, State College of Wash., Pullman. Milk Dealer, 38, 12: 153-155. Sept., 1949.

The potential applications of dielectric defrosting in the dairy industry are: (a) rapid defrosting of bulk frozen fruit for ice cream manufacture, (b) dielectric defrosting of frozen milk and cream by equipment and methods described for frozen fruits and (c) sterilization of milk. A published report describes the dielectric heating of milk to 205° F. in 0.067 sec. after which it was vacuum-cooled to 135° F. in 0.2 sec. The bacterial count was found to be below 1% of that of normally pasteurized milk. Butterfat concen-

tration was unchanged, but apparent cream volume was reduced appreciably. Cost estimates run about 0.6¢/qt.

C. J. Babcock

**183. Scale formation and water.** W. F. BENSON, Limex Corp. Indianapolis, Ind. Milk. Dealer, 39, 2: 62-64. Nov., 1949.

Scale formation on and in equipment through which water is moving is one of the major headaches of industry. In most cases the bicarbonate alkalinity is the culprit and exists because of the  $\text{CO}_2$  solution. There is a balanced relation between soluble  $\text{Ca}(\text{HCO}_3)_2$ , gaseous  $\text{CO}_2$  and insoluble  $\text{CaCO}_3$ . Anything that causes a loss of the  $\text{CO}_2$  promotes scale formation. These causes are usually in 1 or more of 3 categories— an increase in temperature, aeration or an increase in alkalinity. The first 2 are physical and the last a chemical means of causing the change. Evaporation is another physical change that is responsible for scale. The composition of water from different parts of the United States is discussed. Dehydrated complex phosphates slowly remove scale from equipment and keep it clean. Just how they work is not known. On clean equipment, a very thin gelatinous film forms and scale does not form on this film.

C. J. Babcock

**184. Questions and answers for new men in refrigeration.** J. D. CONSTANCE, Operating Eng., 2, 12: 42-43. Dec., 1949.

Questions and answers on the fundamentals of compression systems are presented. The review is simplified by a diagram which includes many possible parts of a complete ammonia system. The 2-temperature installation is explained.

H. L. Mitten, Jr.

**185. Today's pipe welding practices.** F. C. FOUTZ, Midwest Piping and Supply Co., Inc., St. Louis, Mo. Heating, Piping and Air Cond., 21, 11: 81-86. Nov., 1949 and 21, 12: 87-88 Dec., 1949.

Various factors in pipe welding practices are discussed.

H. L. Mitten Jr.

**186. Drips and drains important parts of process and power piping jobs.** G. W. HAUGK, Crane Co., Chicago, Ill. Heating Piping and Air Cond., 21, 10: 76-79. Oct., 1949.

Omission of drips and drains for a piping system is an oversight which will cause added costs. Suitable drains should be provided to drain condensate from all sections of piping and equipment where it may collect. Drains also should be provided for emptying water lines or equip-

ment containing water. At least 1 valve should be placed in each drain line.

Draining condensate from steam lines to auxiliaries before starting unit reduces wear on seating surfaces of control valves and eliminates possible damage from water slugs. The draining of lines adjacent to valves, draining valves in boiler leads, draining of steam headers, drainage of long lines, rises in steam lines and drips for superheated steam lines are discussed and illustrated.

Justification for stress laid upon drips and drains has been proved in practice. Adequate drainage is a safeguard, improves operations and is profitable investment.

H. L. Mitten, Jr.

**187. How to save power dollars.** P. W. SWAIN. 330 W. 42nd St., New York 18, N. Y. Operating Engineer, 2, 10: 19-34. Oct., 1949; Power, 93, 10: 71-86. 1949.

Numbered items which may affect power costs are discussed very briefly. Divisions are steam generation, power generation by steam engines and turbines, steam distribution and application, diesels, gas engines, water power, mechanical-power transmission, air conditioning and heating, electricity and elevators, refrigeration, water services and compressed-air systems.

H. L. Mitten, Jr.

**188. Combustion control.** B. G. A. SKROTZKI, Power, 330 W. 42nd St., New York, N. Y. Power, 93, 12: 71-106. Dec., 1949.

A review of combustion control equipment is presented. It is illustrated with 88 drawings and diagrams which aid in explaining the nature of control and the details of controls made by various manufacturers.

H. L. Mitten, Jr.

Also see abs. no. 213, 227, 242.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**189. Controlling wastage in milk plants.** H. HELMBOLDT. Can. Dairy Ice Cream J., 28, 10: 29-30, 70. Oct., 1949.

Wastage in milk plants can be controlled by: (1) having a butterfat accounting system, (2) eliminating wastage in miscellaneous items like washing powder, caustic, sanitary gaskets, fuel oil, chlorine, mops, dairy brushes, coveralls, gloves, paint, paper towels, hand soap and bottles in stock room, (3) eliminating wastage in bottle caps and hoods, (4) having an inventory control book, (5) having control of sewage disposal, (6) saving in hot and cold water, (7) savings in heat, (8) having proper plant maintenance and (9)

having proper training and cooperation of all men working in the plant. H. Pyenson

**190. Elimination of waste through work simplification.** H. G. DUNLAP. *Can. Dairy Ice Cream J.*, 28, 11: 70-72. Nov., 1949.

The object of any company is to eliminate but not to control waste. The thing to emphasize in a training program include waste of time, energy, materials, products, equipment and space. Time is wasted through poor planning, no planning or not planning far enough ahead. Energy is saved through work simplification training. The posting of material loss helps to cut down plant losses. Product loss can be cut down by elimination of leaky valves and fittings. Supervisors should know capacity of all equipment under their control. Efficient use of space and shelves helps to eliminate waste. Disorder is the lack of time, energy, materials and space and is the main cause of waste. Dirt is usually the accumulation of material not needed.

H. Pyenson

**191. A method of double checking milk samples.** W. W. FOSSETT, Sacramento, Cal. *Milk Dealer*, 39, 2: 72-73. Nov., 1949.

A balance sheet similar to that used in banks is suggested. On one side of the balance sheet would be the fat credited to the patrons for a period of time. On the other side would be the total fat received by the dairy plant for the same period, based on tests made independently from those which determined the fat credited to the patrons. These 2 items should balance within the limits of error which need not be greater than 0.5% of the total amount of the fat handled over the period. Two samples are taken from each weighing, a plant composite and a patron composite. Since sampling is the crux of the system, each sample must be an aliquot portion of the quantity weighed.

C. J. Babcock

**192. Economic operation of an ice cream plant.** R. WISE. *Can. Dairy Ice Cream J.*, 28, 10: 31-34. Oct., 1949.

Economic operation of an ice cream plant depends upon elimination of loss of product, leaks, poor drainage, dirty boilers, oil or air in refrigeration systems, inadequate lubrication, abuse of equipment, irregular maintenance and insufficient records. Over-production of merchandise is not nearly as serious as under-production of new ideas.

H. Pyenson.

**193. Economic operation of an ice cream plant.** ROBERT WISE, Nat'l. Ice Cream Co., East Boston, Mass. *Sou. Dairy Prod. J.*, 46, 6: 96, 98-99, 104. Dec., 1949.

The following suggestions for the economical operation of an ice cream plant are made: Quality and output consciousness of workers, utilization of labor-saving and fatigue-eliminating conveniences, planning of operations in advance, few changeovers, use of sugar in syrup form, simplified equipment, laboratory control of operations, inventory control to have on hand at the proper time all items in proper packages, economical use of ice cream storage space, efficient modern refrigeration equipment, well-designed refrigeration truck bodies with compressor hold-over plates and stainless steel finish, 2-5 deliveries/wk. with cash-on-delivery policy, good public relations from supplying good products in desired packages at fair prices, sanitary control, rodent and insect elimination, safety program, pleasant supplier relations, purchases at low prices but not in excessive quantities, conservative purchases of equipment and ingenuity and inventiveness of the manager.

F. W. Bennett

**194. Cutting materials handling cost in the ice cream plant.** Anonymous. *Ice Cream Rev.*, 33, 4: 39, 54, 56, 58. Nov., 1949.

Present manual methods of handling materials in many ice cream plants are time consuming, expensive and a hazard to the health of workers. Modern methods, involving lift trucks and pallets or skid platforms for materials handling, are being used in a number of large ice cream plants at the present time with complete satisfaction. Many users of this system estimate that the installation cost of trucks, pallets and skid platforms can be recovered within a period of 90 d. through the savings effected.

New developments in the field of materials handling of interest to the ice cream manufacturer are: (1) single service pallets made of paper which can be shipped out with the load by truck or rail and need not be returned, (2) trucks with load grab arms which can be used for handling materials without pallets, (3) stacker for ice cream cabinets on racks which facilitates handling of the cabinets and effects a considerable saving in storage space, (4) a stacker equipped with a stainless steel dumping hopper for dumping sugar into vats.

W. J. Caulfield

**195. Cost of processing and distributing milk and ice cream.** L. C. ANDERSON. *Can. Dairy Ice Cream J.*, 28, 10: 38-42. Oct., 1949.

A table gives the costs of individual products for processing expense, delivery expense, selling expense and administrative and general expense.

H. Pyenson

**196. Bookless bookkeeping.** J. A. GRUNDY,

Remington Rand, Inc. Milk Dealer, **38**, 12: 48-49, 90-92. Sept., 1949

A description is given of the multi-matic Accounting Board System which consists primarily of a board with a movable gripping arm to hold the journal, ledger and other sheets and carbon papers, color and check devices and cards with movable celluloid signal tabs to call attention to such things as overdue accounts, stock shortages, etc.

A journal sheet, the basic bookkeeping document, is inserted in the multi-purpose accounting board. Next, the carbon paper, ledger cards and statement forms are added, for creating 3 bookkeeping records in 1 operation. Posting of identifying information and charge amounts or other data has to be in the right columns, thanks to the color and number guides at the head and base of the board. With 1 part of the posting operation completed, the holding bar or arm is shifted to the next position. Filling in of billing amounts on 1 form also serves to create the basic control information for the customer's ledger card and the journal sheet.

C. J. Babcock

**197. Variables make "Fleet cost per mile" an undependable yardstick.** A. E. FRIEDGEN, A. E. Friedgen, Inc., N. Y. Milk Dealer, **39**, 1: 44, 104-108. Oct., 1949.

A chart is given showing the operating costs per mile per period of 4 wk. of a single fleet. Fleet costs are influenced by variable weather conditions, summer resort business and other factors such as sporting events, horse races and conventions. Any fleet operator who does not get detailed costs for each truck for each period works in the dark. Costs cannot be efficiently controlled by any such general yardstick as fleet cost per mile.

C. J. Babcock

**198. Reducing delivery expense.** J. C. BEDFORD, Armstrong College, Berkeley, Cal. Milk Dealer, **38**, 11: 43, 83-84. Aug., 1949.

Seven points are discussed which can serve as a guide to milk dealers in reducing the operating costs in the service shop and at the same time assure a fleet of trucks on the routes every day at a minimum of expense. They are: (a) check all trucks daily, (b) charge supplies to each truck, (c) replace out-of-date trucks, (d) periodically check all trucks, (e) keep a perpetual inventory of supplies, (f) maintain repair kits by makes of trucks and (g) keep the service shops clean.

C. J. Babcock

**199. Ice cream on milk routes.** Anonymous. Milk Dealer, **38**, 11: 44, 105. Aug., 1949.

Based on the experience of the Polk Sanitary Milk Co. of Indianapolis, Ind., a milk dealer must have enough routes to cover a city completely if he is to distribute ice cream on milk routes successfully. Polk distributes ice cream 3 times a week and offers 4 flavors in container sizes not less than a pint. The price is approximately the same as store prices. Canvas packers did not stand up. Metal boxes insulated with 1 in. of cork, with outside dimensions 28 x 14 x 14 in., holding approximately 10 gal. of ice cream and 2 lb. of dry ice as the refrigerant are used. The ice cream is packed in a specially designed wire basket which can be pulled up so as to give ready access to the entire contents. Ice cream has given the company an added item on the route to help carry overhead and enable the route man to earn a more satisfactory commission. C. J. Babcock

**200. Where can profits in the drug store be increased?** H. H. ROBBINS, Paraffined Carton Research Council. Sou. Dairy Prod. J., **46**, 6: 18, 62-65. Dec., 1949.

The operations of 8 independent drug stores located in neighborhood shopping districts in separate cities in different parts of the country are the basis of the following data. The soda fountain accounted for 39.2% of the stores' sales transactions. Written prescriptions led the other departments in both gross and net earnings. Soda fountains in the group of stores showed an average net profit of 1.7%. The average of profits of 4 profitable fountains was 12.4%, while the 4 unprofitable fountains suffered an average loss of 9.1%. Data on costs and transaction selling times for various items are presented. F. W. Bennett

**201. Design and color in life of your package.** C. H. WHITIS. Can. Dairy Ice Cream J., **28**, 9: 48-50, 52. Sept., 1949.

The pull of color is one of the greatest powers on earth. If used correctly it will increase production by increasing sales. It is not possible to separate packaging from production any more than it is possible to separate merchandising from packaging. H. Pyenson

**202. Food consumption trends in the United States.** B. A. CAMPBELL. Can. Dairy Ice Cream J., **28**, 10: 48-54. Oct., 1949.

Consumption of milk products increased 23% from 1942-1948. The per cent of the weekly earnings spent for food was less for higher income groups. The weekly expenditure by items showed that milk and other dairy products, butter excluded, accounted for 15.8% of total food expenditure for an average family of 2.98 persons.



Butter took another 3% of weekly expenditure. For dairy products there is a gradual increase in consumption as income increases. Average per capita consumption of fluid milk and cheese showed a steady increase as income increased. Consumption of ice cream increased and consumption in the highest income group was 3.5 times the average in the lowest group. Consumption of evaporated milk showed a reverse trend to most other dairy products, with consumption declining as income increased. H. Pycenson

**203. Employee pension plans.** H. E. NYHART, Indianapolis, Ind. *Milk Dealer*, 39, 1: 47, 80-84. Oct., 1949.

The biggest step yet taken toward maximum co-operation between employer and employee is to show the employee that he belongs to the organization by adopting a properly designed retirement plan. A pension plan should be actuarially sound, practical, flexible, attractive and profitable to both employer and employee. A retirement plan is profitable because it makes possible the graceful elimination of superannuated employees who no longer render efficient performance, tends to reduce labor turnover, enables a company to attract and hold a higher type of employee on a career basis and improves the productive efficiency of employees. C. J. Babcock

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**204. Observations on the calcium-phosphorus requirements of dairy cattle.** I. R. JONES, J. R. HAAG, J. H. BYERS AND P. H. WESWIG. Oregon State College, Corvallis. *Proc. 30th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc.*, pp. 69-72. 1949.

The results of preliminary studies failed to reveal a significant relationship between the phosphorus levels of the whole blood of dairy cows and either the stage of lactation or the level of milk production. No correlation was observed between rate of consumption of free-choice minerals (bone meal and disodium phosphate) and level of milk production. N. L. Jacobson

**205. Experiments in rearing calves without whole milk and with limited amounts of skim milk.** H. T. CONVERSE, Bur. of Dairy Ind. U.S.D.A. Cir. no. 822. Sept., 1949.

This publication reviews some of the literature of experiments on raising calves with limited amounts of milk and gives results of experiments conducted on this subject over a period of years at the U.S.D.A. Beltsville Station. A method is

outlined for raising calves without salable whole milk and with only 200-400 lb. of skim milk. The calves are fed a mixture of home grown grains and good-quality, fined-stemmed leafy alfalfa hay. Two to 4 teaspoons daily of cod-liver oil is fed with the skim milk to supply vitamin A until the calf is eating about 1 lb. of hay daily.

R. N. Davis

**206. Once-a-day versus twice-a-day feeding for dairy cows.** J. R. DAWSON AND D. V. KOPLAND. U. S. Dept. Agr. Circ. 830. 7 pp. 1949.

Two groups of 5 cows each received grain, alfalfa hay and corn silage at a morning feeding, or divided equally into a morning and an evening feeding for 3 50-d. periods (by the double reversal method) and were milked twice daily. The grain (barley, oats, mill feed and soybean meal) provided 15.3% of digestible crude protein and 76.9% T.D.N. Each cow received 1 lb. of grain/4 lb. of milk, 30 lb. of silage daily and a hay offering to allow only a 10% refusal. Records for the first 10 d. of each period were discarded as preliminary.

When fed twice daily, cows ate 10% more hay, produced 6% more milk and required 70% more labor for feeding than when fed once daily—small differences distinctly favoring twice-a-day over once-a-day feeding. R. B. Becker

**207. Seasonal adaptation of three methods of curing and storing grass and legume forage as reflected in the milk production of dairy cows.** W. B. LUTZ AND A. R. WOLCOTT. *Mich. Agr. Expt. Sta. Quart. Bull.*, 32, 2: 231-239. Nov., 1949.

Mixed alfalfa-and-quack grass was made into field-cured or barn-dried hay or into field-wilted silage without added preservative. The field-cured hay was 3-wk. more mature at harvest, with a larger proportion of alfalfa. Digestible nutrients were estimated, based on percentages of alfalfa and grass and on chemical analyses. Ten Holstein cows, divided into 3 groups, were fed the respective roughages in 7-d. preliminary and 15-d. experimental periods, with only salt and water extra. Feed of each lot was changed in 3 consecutive periods. Twenty-five per cent more 4% fat-corrected milk was produced on the silage cut in late June than on field-cured hay cut in Aug., and 12% more than on barn-dried hay cut in mid-July. R. B. Becker

Also see abs. no. 159.

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

**208. Breeding efficiency in the Michigan State College dairy herds.** R. C. LEWIS AND R. E. HOB-

wood. Mich. Agr. Expt. Sta. Quart. Bull., 32, 1: 152-155. Aug., 1949.

Breeding efficiency affects calving interval, lactations per cow, season of calving and young stock available, with an assumed ideal of a 10-mo. lactation and 2-mo. dry period per cow. Sixty-five bulls of 5 breeds were at East Lansing, in natural and artificial service, while 11 Holsteins at Chatham and 17 Guernseys at the Kellogg sub-station were in natural service only. In natural use, 2.11 services were required per conception, and 2.63 in artificial use, varying with breeds. The differences were not considered breed characteristics, however, from the limited data. R. B. Becker

**209. Effect of inbreeding on body size, anatomy and producing capacity of grade Holstein cows.** W. W. SWETT, C. A. MATTHEWS AND M. H. FOHRMAN. U. S. Dept. Agri. Tech. Bull. 990. 1949.

Seventy-one grade Holstein cows, for which measurements of body size, anatomy and producing capacity were available, were divided into 4 groups on the basis of the intensities (coefficients) of inbreeding. Analyses of available data were made to determine the effect of inbreeding on magnitude and variability of more than 30 items representing measurements of body weight and skeletal size, size of organs and endocrine glands and milk and butterfat production.

Inbreeding resulted in declines, decreases or reductions in body weight or mass, weight of internal organs (notably the heart), size of endocrine glands (except thyroids), weight of udder, milk production, butterfat production and size of the cows. Inbreeding resulted in increases in the weight of lungs and thyroids, the relation of capacity to weight of udder and proportion of organs and body parts as related to total animal structure. Inbreeding caused little or no significant change in skeletal size, length of intestines, size of pineal and adrenal glands and udder capacity. Inbreeding apparently did not decrease variability in body mass, skeletal dimensions, internal organs, milk production and butterfat production. Inbreeding caused high variability to be observed in the endocrine gland items and in weight and capacity of udders. J. W. Stull

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**210. What becomes of the calves in purebred dairy herds?** R. E. HORWOOD AND E. WEAVER. Mich. Agr. Expt. Sta. Quart. Bull., 32, 1: 149-151. Aug., 1949.

Ultimate disposal of living male and female calves of 5 dairy breeds born in Michigan State College dairy herd over 19 yr. was tabulated. Of 598 heifers and 610 bulls born alive, 77.8% of heifers entered the milking herd, 11.2% were sold for dairy purposes, 2.5% were vealed or slaughtered and 8.3% died, while 53.4% of the bulls were retained or sold for breeding, 38.4% were vealed or slaughtered and 8.2% died. Proportions sold varied with breeds according to local demands. R. B. Becker

**211. Losses of calves in dairy herds.** E. WEAVER, R. E. HORWOOD AND E. S. SMILEY. Mich. Agr. Expt. Sta. Quart. Bull., 32, 1: 42-47. Aug., 1949.

Calf records were analysed from Michigan State College herd over 16 yr.; 9 yr. at Chatham and at the Kellogg Farm Guernsey herd. Of 1,467 calves born, 5.5% were dead (including abortions), 1.1% died at birth and 7.1% died within 10 mo. Losses ranged between 23.6 and 26%, reduction in losses accompanying segregation, feeding from nipple pails, expanded steel calf mats and sulfa drugs when needed. Thirty per cent of the deaths occurred in the 1st wk., and 39.4% more before the 3rd mo. of age. Mortality decreased with age. The herd was brucellosis-free. R. B. Becker

**212. An estimate of the quarterly calving rate of heifers in Welsh counties and the percentage annual replacements in the principality. Part III. The method of calculation.** R. PHILLIPS, University College of Wales, Aberystwyth. British Vet. J., 105, 11: 415-421. Nov., 1949.

Methods are described in the calculation of calving rates in Welsh counties. The calculations involved the 3-yr. totals of calvings of in-calf heifers, yearly total of calvings from the 3-yr. total and the quarterly totals of calvings from the yearly and 3-yr. totals supplemented by returns from the Ministry. The methods of calculation are illustrated by examples and tables.

B. B. Morgan

**213. Design of a milk house.** J. S. BOYD. Can. Dairy Ice Cream J., 28, 10: 62-66. Oct., 1949.

A good milk house will save time and labor on a dairy farm and will make the production of high quality milk easier. The essential equipment of a milk house includes: (a) a milk cooler, (b) a double wash vat, (c) a water heater, (d) a can rack. A dairy producing 6-8 cans of milk should have a minimum of 100 ft.<sup>2</sup> in the milk house, which should be located as close to the barn as possible. Locating the milk house in the barn is

desirable if there is no direct opening to the stable, if a tight wall is constructed between the milk house and stable and if the location is approved by the inspector buying the milk. H. Pynson

Also see abs. no. 132, 134, 206.

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**214. Acidity in ice cream mixes.** W. A. KRIENKE, Fla. Agr. Expt. Station, Gainesville. *Ice Cream Field*, **54**, 4: 96, 128, 129. Oct., 1949.

Titratable acidity is used more commonly by the ice cream industry as an index of quality than is pH. Several modifications of the original Manns acidity test are outlined and the necessity for standardization of procedure is indicated. It is easy to standardize the procedure used in the acidity test, except for the determination of the exact "pink end point." Ability of individuals to detect the "end point" varies considerably. Proper illumination during titration is stressed, also. It is difficult in some cases and may be impractical in the case of colored mixes to depend upon seeing the development of a pink color at the proper end point where phenolphthalein is used as the indicator. The acidity test, as performed in some commercial plants has little or no value. In such cases it would be better to depend upon taste, rather than acidity test, as a measure of quality. When properly performed, titratable acidity tests using phenolphthalein as an indicator give reliable results and "electrometric titration extend the usefulness of titratable acidity values to those ice cream mixes that are colored to the extent that the phenolphthalein pink end point is impractical." W. C. Cole

**215. Serum solids concentrates.** W. J. CAULFIELD AND W. S. ROSENBERGER, Iowa State College, Ames. *Ice Cream Field*, **54**, 5: 46, 68, 69, 70. Nov., 1949.

Three low heat skim powders were compared with extra grade non-fat dry milk solids and sweetened condensed whole milk for use in ice cream. The whipping properties of mixes prepared from low heat powders were equal to or better than comparable mixes prepared with extra grade skim powder or sweetened condensed whole milk. No significant differences in body and texture or meltdown quality of ice cream were observed which could be associated with the type of serum solids concentrate used.

Ice cream prepared with sweetened condensed whole milk was superior in flavor to that prepared with the non-fat dry milk solids, but the flavor score of ice cream prepared with low heat powders was consistently better than that pre-

pared with high heat powders. It also was observed that the flavor of ice cream was better when the mixes were compounded with milk and cream rather than when made with cream and water, irrespective of the type of non-fat dry milk solids used. W. C. Cole

**216. Trends in the ice cream industry.** J. H. DUPLAN. *Can. Dairy Ice Cream J.*, **28**, 12: 31-33. Dec., 1949.

Synthetic ice cream made with vegetable fats is threatening the stability of the ice cream industry. Consumers must be convinced that ice cream is a delicious and inexpensive food. The sales tax on ice cream in Canada is a discrimination and should be repealed or the consumption of ice cream will decrease. H. Pynson

**217. Shrinkage, a progressive report.** J. C. LANDO AND C. D. DAHLE, Penn. State College, State College. *Ice Cream Field*, **54**, 5: 42, 44, 71. Nov., 1949.

Results are given of investigations on mix protein distribution and mix protein proteolysis as related to ice cream shrinkage. Formol titration conducted potentiometrically was used as a measure of mix protein proteolysis. In the case of 12 commercially shrunk samples of ice cream studied, 10 showed formol titration in excess of the titration for the control mix of the same nitrogen content. Mixes to which trypsin had been added and then held at 40° F. for periods up to 72 hr. showed little or no shrinkage in the resulting ice cream held in a cabinet at +5° F. Considerable shrinkage did occur, however, with the ice cream made from the same mix and held for a week in a cabinet at +5° F., if the mix were allowed to incubate at 40° F. for a period of 96 hr. before freezing. It is stated that the albumin-globulin hydrolysis did not begin until about 84 hr. of incubation and it was felt that this was of major importance. When natural unaltered albumin-globulin was added properly to ice cream mixes, shrinkage of the resulting ice cream was reduced. W. C. Cole

**218. Formulas for making sherbets with whey on a commercial scale.** F. E. POTTER AND D. H. WILLIAMS, Bureau of Dairy Industry, U.S.D.A., Washington, D. C. *BDIM-Inf.*-88. Jan., 1950.

Four formulas for using different whey products are given, along with general directions for processing the resultant mixes. F. E. Nelson

**219. Consumer clinics.** H. B. GRANT. *Ice Cream Field*, **54**, 4: 80, 120. Oct., 1949.

Consumer preference is more important than manufacturer's preference in deciding the type of

ice cream that should be manufactured and sold. Reference is made to the "Consumer Clinic" created by the Robert T. Smith Dairy Laboratory of Scranton, Pa. and it is suggested that similar clinics throughout the industry would be desirable. The method of evaluating samples used by the Smith Consumer Clinic is outlined. W. C. Cole

**220. Bulk vs. package.** J. H. FRANDSEN, A. SHIPLEY AND D. H. NELSON, Univ. of Mass. Amherst. *Ice Cream Field*, 54, 5: 38, 40, 65-67. Nov., 1949.

The authors point out certain advantages of packaged over bulk ice cream. Factory-filled packages have a distinct advantage so far as sanitation is concerned. Prevention of shrinkage loss and saving of time during dispensing also are mentioned as advantages of factory-filled packages. Shrinkage losses as a result of dipping or hand filling packages increased from 33.9% with 80% overrun ice cream to 42.3% with 100% overrun ice cream and to 46.4% with 120% overrun ice cream. The body and texture score of freezer packaged ice cream stored at a low temperature was 1.5-2 points higher than ice cream packaged by hand at a higher temperature. W. C. Cole

**221. England's ice cream industry.** C. W. ENGLAND, C. Y. STEPHENS, Dairy and Poultry Industries, Washington, D. C. *Sou. Dairy Prod. J.*, 46, 4: 144, 146. Oct., 1949.

Ice cream is considered a luxury and it is necessary to buy an established business in order to manufacture ice cream as no additional allocations are permitted. Fat is supplied from oleomargarine, sweet fat and evaporated milk. Sources of serum solids are skim milk powder, Syrol, evaporated milk and sweet fat. Sweet fat is 30% oleomargarine fat, 50% sugar, 15% skim milk powder and 5% dextrose. Syrol contains 84% lactose, 16% albumin and milk salts and apparently is from cheese whey. The finished product contains approximately 31% T.S.

All equipment is of English manufacture, only the pasteurizer differing radically from that used in the U. S. The pasteurizer is of the water-jacketed type, heated by a gas burner at the bottom, and the pasteurizer also is used for cooling.

A part of the water, the ice cream powder, Syrol and skim milk powder are heated in the pasteurizer to 195° F. Additional water is added and the mix cooled to 160° F., starch digesting enzymes (bacterase) added and held for 10 min. The remainder of the ingredients then are added and the complete mix pasteurized at 160° F. for 30 min., homogenized and cooled.

Restrictions on the use of whole milk for ice cream making were lifted for the summer only.

F. W. Bennett

**222. Holland's dairy and ice cream industries.** C. W. ENGLAND, C. Y. STEPHENS, Dairy and Poultry Industries, Washington, D. C. *Sou. Dairy Prod. J.*, 46, 5: 60, 62, 64, 66. Nov., 1949.

The annual/capita consumption of frozen dairy products is about 1/10th of the annual consumption in the U. S. The principal product manufactured by most of the ice cream plants is similar to our ice milk. Available ingredients include milk, cream, skim milk powder, allocated sugar, sterilized milk, imitation egg white, gelatin, sodium alginate, carboxy methyl cellulose, emulsifiers and vanilla flavor which lacks fine character. Mix is pasteurized by the holder method and has a negative Storch test. Most of the freezers observed were vertical brine machines. Overrun is usually 20-40%. Much ice cream is sold in bars made in 1-1 brick pans. Holland has a very active retail ice cream manufacturers' association.

Milk received from farms averages 3.3% fat and is standardized to 2.5% fat for retail. There are 3 grades of milk: bottled milk, pasteurized at 167° F. for 13 sec., loose milk, pasteurized at 185° F. for 2 sec. and sterilized milk, heated under pressure to 239° F. for 30 min. and bottled in qt. beverage bottles with metal crown caps.

Sanitary stainless steel pipe lines usually are installed permanently. Pipe lines and HTST pasteurizers are cleaned by circulation with steam and chlorine used for sterilization. Results of bacteriological checks are surprisingly good. Practically all equipment is of European manufacture. Plant construction is much the same as in the U. S.

F. W. Bennett

Also see abs. no. 173, 176, 192, 193, 194, 195, 199, 200.

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**223. "Flavor insurance" for your bottled milk.** S. J. WEESE AND KYLE WELLS, West Virginia Univ., Morgantown. *Milk Dealer*, 39, 2: 58-59. Nov., 1949.

Flavors and odors present in the atmosphere of home refrigerators are of great practical significance in affecting changes in flavor of milk held in these refrigerators in bottles without caps. The flavor absorbed may be very strong and typical of the material from which it originated. Using different fruits and vegetables, cucumbers gave the milk the lowest flavor score, followed by onions, honeydew melons and strawberries.

C. J. Babcock

**224. Comparison of raw milk grades as determined by the methylene blue test and the sediment test.** J. C. BOYD, Idaho Agr. Expt. Sta. and

LESTER HENDRIX, Idaho State Dept. of Agr. Milk Dealer, 38, 12: 64-68. Sept., 1949.

The effectiveness of the sediment test as compared with the methylene blue test in detecting milk of poor quality was studied. In conducting the methylene blue test the samples were not inverted after the milk and dye were mixed. Comparisons of the methylene blue test and the sediment test on 602 individual cans of milk from 5 representative milk processing plants showed 26% of the milk sampled to be of poor sanitary quality (decolorized methylene blue in 20 min. or less) and that 14.5% of the milk sampled contained excessive amounts of sediment. No correlation was found between the milk containing excessive amounts of sediment and that which reduced methylene blue in 20 min. or less. Much of the milk containing excessive amounts of sediment is not detected by the methylene blue test. Neither the sediment test nor the methylene blue test, when used alone, is entirely effective in determining whether milk should be accepted. The routine use of both the sediment test and the methylene blue or some similar quality test would be very helpful in a milk grading program in those areas where milk is being produced for the manufacture of butter, cheese, evaporated milk, milk powder and other products. The use of some other quality test, along with the sediment test in those areas is strongly recommended. C. J. Babcock

225. **Going grade A.** E. THOM. Milk Dealer, 38, 12: 45-46, 108-116. Sept., 1949.

A description is given of how the milk dealers in Evansville, Indiana, obtained the cooperation of the Extension Service of Purdue University, county agents, health authorities, consumers, city officials and others in promoting a Grade A milk program. The construction costs for converting Evansville area farms into Grade A units has been between \$300 and \$1,100, the amount varying depending upon how much work the farmer does himself. The results of the program are shown by the fact that in 1945 not 10 producers in the area could have qualified under the U. S. Public Health Service Grade A program. Today, 475 are qualified and from 200 to 250 additional producers have done some necessary remodeling. When the program was first introduced, 100 marginal producers not seriously in the dairy business dropped out of the market rather than comply with requirements. C. J. Babcock

226. **Tank trucks for farm pick-up of milk.** G. E. SARTAIN, Bryant and Chapman Dairy, Hartford, Conn. Milk Dealer, 38, 11: 64-69. Aug., 1949.

As of April 1, 1949, the Bryant and Chapman

Dairy had installed tanks on 6 farms. The farm tanks vary in size from 200-600 gal. and are rectangular in shape. They are owned by the company and the producer pays a small rental for their use. The tanks are equipped with coolers that reduce the temperature of the milk from 95° to a range of 55-38° F. within 5 min. The milk is mechanically agitated until it reaches 38° and then the compressor and agitator shut off. They automatically start if the temperature of the milk rises to 42° F. The milk is agitated and samples for testing taken before being pumped into the pick-up 2-compartment insulated tank. The milk pump driven by a power take-off from the truck engine is located on the truck. The milk is pumped through a heavy gauge brewer hose with sanitary fittings. The farmers who now have these tanks installed feel that this is the first time in the history of marketing milk that a new idea and practice has been so beneficial to both the farmer and his distributor. C. J. Babcock

227. **Equipment problems and efficiency of operation.** H. PUTNAM. Can. Dairy Ice Cream J., 28, 11: 80-84. Nov., 1949.

The efficiency of equipment operation in a fluid milk plant is given and means of solving equipment problems are listed. Employees and foreman should thoroughly understand the machine in new installations. Plant operating conditions should be examined from the standpoint of obtaining an orderly operation. Mechanical agitators in weigh cans properly operated assures a more uniform mixture of the product than some other methods of agitation. Composite samples should be stored in a mechanical refrigerator. In planning equipment efficiency, the following equipment should be given consideration: sanitary pumps, mechanical can-washing, storage tanks, ice builders, water cooling, evaporative condenser, steam plant, bottle washer, bottle filling, H.T.S.T. pasteurizer and homogenizer. H. Pyenson

228. **Lift trucks.** Anonymous. Milk Dealer, 38, 11: 48, 90-99. Aug., 1949.

Lift trucks save manpower and money for milk dealers. They are being used at several plants. At the Whiting Milk Co. in Charlestown, Mass., 36 cases are loaded on a pallet for storage in the milk cooler and are moved out of the cooler to delivery trucks, still stacked on the pallets, by means of lift trucks. The general results of handling loaded milk cases and empties at the Whiting plant are as follows: (a) space required for semi-trailer parking was reduced by two-thirds; (b) trailer hold-up time was cut down from 40 min. with 6 men to 30 min. with 1 man; (c) number of semi-trailers required for moving the loads

over the streets was reduced from 5 to 2 and breakage of bottles was reduced considerably; (d) a big advantage to this system is that it saves floor space.

The size and capacity of the lift truck selected depends upon maximum weight and maximum size of loads to be carried, *i.e.*, plant floor conditions (inclines or ramps, elevators, aisles, doorways, etc.) and the size and capacity of trucks now in use.

C. J. Babcock

**229. Whipped cream in upstate New York.** A. C. DAHLBERG AND F. V. KOSIKOWSKY. *Can. Dairy Ice Cream J.*, **28**, 9: 35. Sept., 1949.

Most cream mixes were whipped by instantaneous release of the mix from the pressure of  $N_2O$ . This instantaneously whipped cream generally contained about 20% butterfat. It often contained a small amount of added nonfat milk solids. The sugar content averaged about 6% and vanilla flavoring usually was present. About 400% overrun was obtained per pint container. The whipped cream prepared by mechanical agitation contained 29% butterfat, and 7% sugar and the overrun averaged slightly below 200%. The creams whipped instantaneously by  $N_2O$  gave av. logarithms counts of 299,000 bacteria/ml. and 193 coliform bacteria/ml. Mechanical whipped samples averaged in the millions for total bacteria.

H. Pyenson

**230. Ten traits that make a stream-lined salesman.** M. O. MAUGHAN, Northwestern Univ. *Milk Dealer*, **38**, 11: 51, 98-102. Aug., 1949.

The 10 most important characteristics are: (1) he builds confidence, (2) he sells benefits, (3) he talks facts which are built on a thorough knowledge of his merchandise, (4) he practices showmanship, (5) he renders definite and tangible service during each interview, (6) he practices creative selling, (7) he emphasizes quality, (8) he constantly grows and develops himself, (9) he knows the art of dealing successfully with people and (10) he uses system in his presentation. A description of how a salesman can present his case in 5 talking steps as well as 7 possible methods of closing are discussed.

C. J. Babcock

Also see abs. no. 147, 169, 189, 191, 195, 198, 199.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**231. Some studies of the circulatory system of the cow's udder.** W. W. SWETT AND C. A. MATTHEWS, U. S. Dept. Agr. Tech. Bull. 982. 36 pp. June, 1949.

Correlations between udder veining, milk veins,

milk well size and production records of 106 Holstein-Friesian and 89 Jersey cows at Beltsville, Md. were low and considered insignificant. Extensive veining tended to occur on the surface of tight, compact, closely attached udders more frequently than on loose and flexible ones.

Arteries were injected with red liquid latex and veins with blue liquid latex, hardened by injecting a solution containing 2% of acetic acid and 10% in formalin which hardened the latex, so that tissues could be dissected away. Impressions of valves indicated direction of blood flow. Tissues were dissected away from the larger vessels in 1 udder, while 25 udders were sectioned for observation. Frequent anastomoses were found between arteries and between veins within the separate halves of the udder. Transmedian anastomoses were found between veins of 12 udders in front of the fore teats, in 5 between the fore and rear teats, and in 10 udders in the rear portion, including those between the perineal veins. Fewer transmedian arterial anastomoses were found. Major arteries and veins were described. Main entry of blood was through the external pudic artery in each half of the udder. Both veins and lymph vessels occurred as the surface vessels on udders of 17 living cows.

The external pudic and subcutaneous abdominal veins carried venous blood outward. All valves in the perineal vein, except in immediate proximity to the vulva, pointed toward the udder, indicating blood circulation in that direction.

R. B. Becker

**232. Weight and capacity of the dairy cow udder in relation to producing ability, age and stage of lactation.** C. A. MATTHEWS, W. W. SWETT AND M. H. FOHRMAN. U. S. Dept. Agr. Tech. Bull. 989. 1949.

Data on the udders of 473 cows (Holsteins, grade Holsteins and Jerseys) were used in numerous group and correlation studies to determine the relationships between udder size and producing ability and the effect of age, breed, stage of lactation and length of dry period on the weight and capacity of the udder. Group averages and correlation studies showed definite increases in udder weight and capacity with age. Advance in the stage of lactation was associated with variable but definite decreases in udder weight and less definite decreases in capacity. Increases in the length of dry period were associated to some extent with decreases in udder weight and capacity.

Highly significant correlations between producing ability and udder weight and capacity were found in both groups of lactating cows and in both groups of dry cows. The ratios calculated by

dividing udder capacity by empty weight of udder had no consistent significant relationship within all groups with the effects of age, lactation cycle or producing ability. However, there were significant positive correlations between this ratio and advance lactation for cows lactating 2 mo. or less and cows lactating over 2 mo.

J. W. Stull

**233. Weights and capacities of udders from dairy heifers of different ages.** C. A. MATTHEWS, W. W. SWETT AND M. H. FOHRMAN. U. S. Dept. Agr. Tech. Bull. 993. Nov., 1949.

Studies of empty weight were made on udders from 71 heifers, and studies on capacity were made on 42 syringe-filled udders and 54 pressure- or gravity-filled udders. Weights of udders of heifers 3-30 mo. of age increased at an average of approximately 0.59 lb./mo. Capacities of 33 pressure- or gravity-filled udders from heifers 9-30 mo. increased at an average of approximately 0.44 lb./mo.

Capacities of 36 syringe-filled udders between birth and 9 mo. of age increased definitely but at a rate out of line with that calculated from pressure- or gravity-filled udders. Heifers over 30 mo. of age and heifers in which pregnancy had terminated early in the gestation period had udders lower in average weight and capacity than heifers 24-30 mo. There was no definite trend with age in the ratio of capacity to weight in udders from heifers 9-30 mo. of age. The average ratio of capacity to weight was lower for udders from heifers 24-30 mo. old than in younger heifers and considerably lower for heifers over 30 mo. and heifers once pregnant.

R. N. Davis

## NUTRITIVE VALUE OF DAIRY PRODUCTS

R. J. JENNESS, SECTION EDITOR

**234. Nutritive value of milk and milk products sold in Montreal.** M. BLAIS, R. BEARDON AND M. SANDERSON. Can. Dairy Ice Cream J., 28, 11: 110-114. Nov., 1949.

Ca and P content of fluid milks sold in Montreal over a 9-mo. period averaged 109-127 mg. % for Ca, with a mean value of 114, and from 86-94 mg. % P with a mean of 88. Pasteurization and homogenization did not effect the content of these minerals in fluid milk. Chocolate dairy drink contained less Ca but more P than milk. Butter-milk had slightly greater amounts of Ca and P than raw milk. Ice cream is a moderately good source of Ca, but not as good as fluid milk when considered on a per serving basis.

H. Pynson

**235. The chemistry of fats in relation to their nutritional significance.** E. L. JACK AND L. S. OLSEN. Univ. of Cal., Davis. Proc. 30th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc., pp. 42-46. 1949.

This report summarizes some of the more recent research on the dietary interrelationships between fat and other nutrients and on the nutritional significance of the chemical constitution of fats.

N. L. Jacobson

**236. Recent advances in nutrition.** L. A. MAYNARD. Can. Dairy Ice Cream J., 28, 9: 32-33. Sept., 1949.

Recent advances in nutrition are reviewed.

H. Pynson

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**237. Blood levels of certain constituents in normal and spasmophilic calves.** T. H. BLOSSER, G. W. SCOTT, JR., U. S. ASHWORTH, R. E. ERB AND A. O. SHAW, Washington State College, Pullman. Proc. 30th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc., pp. 61-68. 1949.

A disease of dairy calves which the authors describe as spasmophilia is discussed. It is characterized by spasmodic seizures followed by exhaustion and high mortality. Blood analyses which included protein, phosphorus and sugar in the plasma and Ca, Mg, and citric acid in the serum, as well as hemoglobin, failed to reveal the cause of this disorder.

N. L. Jacobson

**238. Quantitative aspects of the diabetogenic and the growth-promoting activities of pituitary preparations.** P. M. COTES, E. REID AND F. G. YOUNG, University College, London, England. Proc. Soc. Endocrinol., 12th Ordinary Meeting, Oct. 21, 1948, pp. 14, 15. (in J. Endocrinol., 6, 2, Oct., 1949.)

Studying ox pituitary extracts, the authors interpreted their results to mean that pituitary diabetogenic and growth-promoting activities are due to the same substance.

V. Hurst

**239. The influence of thiouracil on reproduction and growth in the rat.** S. B. BARKER, Dept. of Physiology, State Univ. of Iowa, Iowa City. J. Endocrinol., 6, 2: 137-144. Oct., 1949.

Thiouracil administration as 0.2% of the ration depressed the reproductive capacity of females and retarded the growth rates of the young. The earlier the young received thiouracil, the more

evident was the ability of thiouracil to retard growth. Rats returned to a normal diet following prolonged periods of thiouracil administration regained their reproductive capacity and produced normal offspring. V. Hurst

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**240. Effect of the condition of the milk can on the microbial content of prepasteurized milk.** N. A. MILONE AND W. D. TIFDEMAN, N. Y. State Dept. of Health, Albany, N. Y. *J. of Milk and Food Technol.*, **12**: 332-347, 369 Nov.-Dec., 1949.

The authors claim there was no appreciable effect on the bacterial content of prepasteurized milk poured into milk utensils that have been properly sanitized and dried. A high thermophilic bacterial count in milk cans may contribute to unsatisfactory reduction efficiencies on HTST or LTLT pasteurization methods. It appears a producer must be very careless with his milk cans in order to grossly contaminate milk poured therein, except in extreme instances, high microbial content of the milk generally was not attributed to the milk can. H. H. Weiser

**241. Bottle washing studies under plant conditions.** C. N. SIARK, R. F. HOLLAND, J. C. WHITT AND M. J. GURDIAN, Cornell Univ., Ithaca, N. Y. *Can. Dairy Ice Cream J.*, **28**, 9: 36, 38. Sept., 1949.

A bacteriologically satisfactory milk bottle can be obtained by using the concentrations of alkali and the corresponding holding time and temperatures suggested by Levine for washing bottles under commercial plant conditions. The preferred soaking temperature is 160° F. Tetrasodium pyrophosphate was essential to obtain satisfactorily rinsed bottles. The addition of trisodium phosphate, in the amounts used, did not improve the washing process. The measurements made on surface tension, specific gravity and carbonate alkalinity in most instances did not correlate with the satisfactory rinsing of the bottles or the number of surviving bacteria found in the bottles. Soaker solution temperatures of 170° F. or higher are not needed to obtain a clean, excellent appearing, low bacterial count bottle. H. Pyenson

**242. Special equipment improves cleaning operation and reduces plant costs.** J. R. PERRY, Sealtest Inc., New York, N. Y. *Milk Dealer*, **39**, 3: 46-47, 89-94. Dec., 1949.

For general rinsing as a part of the cleaning op-

eration, water with a temperature of 115° F. is desirable. This may be supplied satisfactorily by a hot and cold water blending or tempering valve. To obtain satisfactory rinsing, there should be a sufficient quantity of water at the right pressure for the rinsing job to be done. A uniform pressure at every outlet and hose station is not satisfactory. In most plants it is desirable to have available rise water in adequate quantity at all locations and at varying pressures.

A variety of water pressures in a plant can be simply had, provided the entering supply has enough pressure. The water should reach the points of use at a pressure somewhat in excess of the highest pressure needed; in each location where a lesser water pressure is desirable, a pressure-regulating valve and a pressure gauge may be installed. This is not an expensive way to arrange water pressure and the better rinsing as well as the saving of water will justify the small investment in pressure regulators and gauges. A 0.5" special lightweight hose with a whip-end and shut-off valve is recommended.

C. J. Babcock

**243. Detergent-sanitizers to improve milk quality.** M. A. COLLINS. *Can. Dairy Ice Cream J.*, **28**, 9: 45-47. Sept., 1949.

The one-solution detergent-sanitizer in liquid form proved satisfactory for washing and sterilizing metal pails and strainers and for maintaining low bacterial counts in the raw milk. Even in cold water it is unlikely that any milk or chemical film will develop on the metal surfaces with properly washed metal utensils using the detergent-sanitizer. The one-solution method for cleaning and sanitizing utensils and udders is inexpensive. A 140-150 p.p.m. solution of active disinfectant did not irritate the cows teats or the hands of the milker. It prevented the spread of an infection which developed due to injury of one quarter of a cow's udder. H. Pyenson

**244. The use of hypochlorite and quarternary ammonium compounds in the routine washing of cows udders prior to machine milking.** E. M. KESLER, C. B. KNOTT, G. H. WATROUS AND P. S. WILLIAMS, Penn. State College, State College. *J. of Milk and Food Technol.*, **12**: 350-353. Nov.-Dec., 1949.

A comparison was made of the bacterial count on udder washes containing hypochlorite and quarternary ammonium compounds in controlling the plate count of milk obtained by machine milking. When 400 p.p.m. and 200 p.p.m. of quarternary ammonium compound, 400 p.p.m. and 200 p.p.m. chlorine and water alone were used they showed no appreciable differences in the



microbial counts of the milk produced. However, differences among cow groups were found to be significant.  
H. H. Weiser

**245. A new method for the evaluation of quaternary ammonium detergent sanitizer compounds.** G. R. GOETCHIUS AND W. E. BOTWRIGHT, Rohm and Haas Co., Philadelphia. Soc. Am. Bact., Abs. of Papers, p. 56. May, 1949.

To simulate milking machine conditions, sterile rubber strips are immersed in a milk suspension of the test organism. These are allowed to dry partially and then are immersed in the use-dilution of the detergent sanitizer that has been prepared in natural hard water. The sanitizing solution is contained in a beaker placed over a magnetic stirrer. After sanitization, the strips are dipped in tap water and placed in sterile petri dishes which are poured with T.G.E. agar containing an inactivator for the quaternary compound. *Escherichia coli*, *Streptococcus fecalis* and *Pseudomonas aeruginosa* were selected as being most typically resistant to quaternary ammonium germicides. As a control, tap water is substituted for the sanitizing solution. An acceptable detergent sanitizer will show 99% reduction in bacterial count over the control. The laboratory method correlated well with field tests on milking machines and other dairy equipment.

D. P. Glick

**246. Rationalizing the failure of a quaternary ammonium compound to detect true germicidal activity in sanitizing glass containers.** H. E. LIND AND D. ALLAN, Sias Research Laboratories, Brookline, Mass. Food Tech., 3, 9: 304-306. 1949.

Six oz. screw cap bottles were washed in a hot detergent solution, rinsed in hot water and the bottles and caps immersed in a solution containing 100, 200 and 400 p.p.m. of a quaternary ammonium compound (Roccal) for periods of <0.2, 5 and 10 min. After the bottles had drained, 50 ml. of a rinse diluent were added to each bottle and the capped bottles were shaken 25 times; 1-ml. aliquots of the rinse liquid were plated on standard tryptone agar; after 48 hr. incubation at 37° C. the colonies present were counted.

The use of a neutralizer rinse was essential to demonstrate optimum performance of the quaternary ammonium compound. Unbuffered and buffered distilled water, sodium thioglycollate broth and Lethen broth were unsatisfactory as rinse diluents. It was found that 0.2% ascolectin in 2% Tween 80 was very satisfactory as a neutralizer diluent. More than 85% of the bottles in each trial showed a residual plate count of

less than 100/ml. and the percentage of kill/bottle exceeded 99.9%.  
E. R. Garrison

**247. Insect control—The general situation.** H. H. SCHWARDT. Can. Dairy Ice Cream J., 28, 9: 52. Sept., 1949.

Flies have developed a strong resistance to several of the new insecticides such as D.D.T., methoxychlor and chlordane. Continuous development of new insecticides or improvement of older ones must be expected. It is possible that an alternation of insecticides over periods of years will take care of the resistance problem. Toxicity studies will be made on all new insecticides offered for sale from now on. Lindane is the pure gamma isomer of benzene hexachloride. Lindane kills flies faster than D.D.T. but its residual effect is of shorter duration. Eight lb. of 25% wettable powder or 1 gal. of 25% emulsifiable lindane should be used in each 100 gal. of spray. It is safer than D.D.T. since it does not accumulate in the body above the level of daily intake. Where roaches are a problem around milk plants, chlordane should be used.  
H. Pynson

**248. Proper formulation of insecticides in food plants.** H. E. WHITMIRE, Whitmire Research Lab., Inc., St. Louis, Mo. Sou. Dairy Prod. J., 46, 4. 106-107. Oct., 1949.

The possible health hazards of insecticides are more important than the insect control properties. Most pest control materials are not developed for use in food processing establishments. Reactions may occur between certain base oils and insecticidal chemicals, resulting in higher toxicity than possessed by either the oils or insecticidal ingredients alone. Many synthetic organic chemicals have unknown toxic properties. There are toxicity differences between chemically pure and technical grades of insecticidal chemicals. Presumably inert vehicles have been fatal in some instances. Insecticides of known or unknown toxicity which may be hazardous, should not be permitted upon the premises of a food processing plant.

Two proven insecticidal agents are dihydro-rotenone (hydrogenated rotenone) described in U. S. Patent no. 1,945,312 and pyrethrin-like esters developed by R. C. Roark and associates.  
F. W. Bennett

**249. A bug's eye view of dairy plant architecture.** ED. M. SEARLS, National Dairy Products Co., Inc. Milk Dealer, 39, 3: 42, 43, 100-104. Dec., 1949.

A discussion is given of the effect of plant location and construction on insect and rodent con-

trol. The presence or absence of insects and rodents is one of the most readily available and most reliable indices of sanitation. Insect prevention in milk processing plants, previously the unique responsibility of the entomologist, has greatly increased in scope until today it also requires the best cooperative efforts of the dairy products plant architect.

C. J. Babcock

**added chemicals in food.** W. B. WHITE. Can. Dairy Ice Cream J., **28**, 11: 75-79. Nov., 1949.

A survey of the protection afforded consumers against added chemicals in food in the United States is given. Some of the chemicals mentioned are diethylene glycol, ethylene glycol, D.D.T. and other insecticides and fungicides.

H. Pynson

**250. Protection afforded consumer against**

Also see abs. no. 155.



# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

### BOOK REVIEWS

**251. Guide to the Dissection of the Cow.** R. E. HABF1, New York State Veterinary College, Ithaca, N. Y.

This booklet should provide an excellent dissection guide for veterinary students, the purpose for which it is intended. Some parts might readily prove of help to others, such as students of dairy husbandry receiving limited instruction in this field. The brief references to the clinical and surgical importance of various tissues are a helpful addition. For instance, in regard to the obturator nerve, mention is made of the possibility of injury during parturition with resultant paralysis of the adductor muscles. Even more such references might have been included to advantage.

The author is to be commended for the evident thought and care that have been expended in the development of this booklet. With its adequately large type, having the names of the various structures underlined when first encountered, being ring-bound and of moderate size, it should facilitate placing increased emphasis on cattle in veterinary anatomy laboratories.

W. D. Pounden

**252. Outlines of Food Technology.** 2nd ed. HARRY W. VON LOESECKE. 585 p. Reinhold Publishing Corp., 330 W. 42nd St., New York 18, N. Y. \$7.50. 1949.

The revised edition follows quite closely the organization of subject matter presented in the earlier edition published 8 yr. ago. The revision appears to consist essentially of added material, with less emphasis on rewriting of the original discussion. The 15 chapters cover the tin can and glass container, fruits, vegetables, dairy products, meats and poultry, fish products, grain products, fats, oils, sugars and starches, nuts, spices and flavor materials, beverages, confectionary and preserves, freezing and storage and marketing. The author has endeavored to provide a good insight into prevailing practices, and char-

acteristics of products and materials used in the food industries.

He has provided useful information on typical procedures suitable for survey instructional purposes. The section on dairy products is in great need of re-organization and revision in light of proportion, order and obsolescence of material.

K. G. Weckel

**253. Brucellosis.** 2nd ed. H. J. HARRIS. Paul B. Hoeber, Inc., New York 16, N. Y. 617 pp. \$10.00. 1950.

The presentation is largely that of the physician interested in the control of human brucellosis. However, much information on the control of animal reservoirs of the disease is included. The presentation is very complete and very well documented, 742 references, many of them recent publications, being included in the literature cited. A good index is provided.

In addition to extensive coverage of symptomology, diagnosis and treatment of human brucellosis, much material on etiology, epidemiology and prophylaxis is presented. The role of milk and milk products in the transmission of the disease is discussed in detail and pasteurization is advocated as the primary basis for control. A holding period of at least 6 mo. for cheese made from raw milk is considered necessary. The blood test for agglutinins is not considered a satisfactory procedure from the public health standpoint because of the possibilities that animals giving titers below those usually considered as positive may be shedders of the *Brucella* organisms. The role of meats and direct contact in dissemination of the causative organisms also is discussed.

This is a valuable reference book in an area of importance to all in the dairy industry.

F. E. Nelson

**254. Food Poisoning,** rev. ed. G. M. DACK. University of Chicago Press, Chicago. 179 pages. \$3.75. 1949.

Three of the reasons for the many advanced principles of sanitation technology existing in the

dairy industry are the potential vector properties of milk, the wide spread use of milk as a food by people and its use as an ingredient in many food products. Fortunately, food poisoning is not frequently attributed to the use of dairy products because of the sanitation techniques employed. Nevertheless, sanitarians must understand the potentialities, and not lose sight of the principles incorporated in sanitation practice requirements. The book has been revised from the excellent original issue by incorporation of much information gained during World War II. The subject matter is divided into 8 chapters (involving foods) on chemical poisoning, poisonous plants and animals, botulism, staphylococcus food poisoning, Salmonella, alpha-type streptococci and food poisoning, other bacteria and food poisoning and infections differentiated from food poisoning. The characteristics of the various forms of poisoning are interestingly reviewed, case examples are cited and control measures commented upon. The book is very readable. It is one every handler, processor and supervisor of foods should have since it provides an excellent fund of information on this subject.

K. G. Weckel

**255. Advances in Agronomy.** Vol. 1. A. G. NORMAN, editor. Academic Press, Inc., New York, N. Y. 439 pp. \$7.50. 1949.

This volume, prepared under the auspices of the American Society of Agronomy, inaugurates a new series of annual reviews in a field not covered previously. The subjects covered are: Plant growth in saline and alkali soils, by H. E. Hayward and C. H. Wadleigh; New fertilizers and fertilizer practices, by R. J. Jones and H. T. Rogers; Soybeans, by M. G. Weiss; The clay minerals in soils, by J. E. Gieseking; Alfalfa improvement, by W. J. White; Soil microorganisms and plant roots, by F. E. Clark; Weed control, by A. S. Crafts and W. A. Harvey; Boron in soils and crops, by K. C. Berger; Potato production, by O. Smith; and Fixation of soil phosphorus, by L. A. Dean. Each review is accompanied by a table of contents and apparently adequate subject and author indices for the entire volume are included. Those interested in certain aspects of the feeding of dairy cattle will find much of interest in this volume.

F. E. Nelson

## ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**256. Penicillin-streptomycin bougies in the treatment of acute infectious mastitis.** C. S. BRYAN, V. P. LABRANCHE and A. R. DRURY, Mich. State College, East Lansing. North Am. Veterinarian, 31, 1: 20-25. Jan., 1950.

Results of *in vitro* studies showing the influence of penicillin, streptomycin and penicillin

plus streptomycin on strains of streptococci and staphylococci originally isolated from cases of infectious mastitis indicate that streptomycin was effective in inhibiting growth of both of the above; penicillin alone was much more effective and produced the same results as the combination of the two antibiotics.

A second part of this paper involved a study of the irritant effects of these antibiotics using both aqueous and bougie administration. Neither penicillin or streptomycin alone nor the combination of the two produced irritation of the udder tissue as indicated by the leucocyte count of the milk.

The penicillin and streptomycin combination was successful in 1 case of experimentally produced coliform mastitis, 18 cases of chronic streptococcal mastitis, and 9 cases of naturally occurring coliform mastitis. R. P. Niedermeier

**257. The combined use of the Hotis and microscopic tests for mastitis samples.** L. W. VAN DER HUVER, Germiston, S. Africa. J. So. African Vet. Med. Assoc., 20, 1: 16-20. March, 1949.

A comparison between the microscopic and Hotis test for detection of mastitis milk is presented. Negative findings were in agreement in 94.5% of the samples. Of 31 samples which gave questionable microscopic readings, 33% were positive to the Hotis test. The author concludes that the most reliable results can be obtained by using both tests. The Hotis test assists materially in clarifying doubtful microscopic findings.

K. M. Dunn

**258. Determinatie van Mastitis-verwekkende Bacterien (Determination of bacteria causing mastitis).** (English Summary). C. F. VAN OYEN and G. B. R. WILLEMS, Laboratory for knowledge of human food from animal sources of the State University at Utrecht, Holland. Tydschrift voor Diergeneeskunde, 74, 2: 91-96. 1949.

Authors describe the determination of pathogenic bacteria occurring in milk or udder secretion from cases of acute or chronic mastitis in cows. Methods were based on the work of A. F. van der Scheer (thesis 1941, College of Agriculture, Wageningen, Holland). Pure cultures are cultivated first and then the different bacteria, *Streptococcus agalactiae*, *dysgalactiae*, *uberis* or *pyogenes* determined by the way they react on selective media and solutions of different carbohydrates. Selective media were: agar-agar with horse-serum and broth of calfsmeat, meat broth, litmus-milk, gelatin, sodium-hippurate solution, esculin agar, horse blood-agar plate. Carbohydrates were: Saccharose, raffinose, salicin,

mannite, sorbite, trehalose, inulin, amygdalin and arbutin; solutions were made in broth containing Difco-meat extract, Difco-neopepton, horse-blood serum and sodium chloride with bromo-cresol-purple as indicator.

There was a good correlation between these bacteriological determinations and clinical observations.

A. F. Tamsma

**259. Control and eradication of brucellosis in animals. Report No. 1 of the National Research Council, Committee on Public Health Aspects of Brucellosis.** W. W. SPINK, L. M. HUTCHINGS, C. K. MINGLE, C. L. LARSON, W. L. BOYD, C. F. JORDAN and ALICE C. EVANS. J. Amer. Med. Assoc., **141**, 5: 326-329. Oct. 1, 1949.

This special article is an excellent, concise and complete summary of the best knowledge and opinion on the subject. About 5% of the adult female cattle in the U. S. are affected with brucellosis. This involves at least 1,300,000 dairy and 800,000 beef cows. These are confined to about 20% of the herds. The total annual loss from decreased milk production and veal calves, and replacements of dairy cows approximates \$92,000,000. Between 1 and 3% of swine are infected, based on packing house surveys. Unlike other livestock disease problems, no single plan of control has proved effective under all conditions with brucellosis. Confusion has resulted from the ardent sponsorship of the proponents of different plans. Agreement on basic procedures and nationwide acceptance of these procedures are needed.

Twenty-one fundamental items of knowledge about the disease in general are listed. These are too long to reproduce in abstract form but should be studied carefully by all interested persons. Under a separate discussion of bovine brucellosis, 11 procedures are itemized as recommendations for state legislation, including 4 plans for eradication. Educational policies are also discussed.

Swine brucellosis is discussed separately and 2 plans of control are presented. Control and eradication of brucellosis in other animals probably do not require a nationwide uniform program, so essential in cattle and swine, but it should be recognized that other species may be affected and may transmit the disease to other animals and to man. Since man is infected by exposure to infected animals and animal products, the problems are largely those of the veterinarian.

D. P. Glick

**260. A new aid for the control of brucellosis.** G. C. VAN DRIMMELEN, Inst. of Onderstepoort, Pretoria; S. Africa. J. So. African Vet. Med. Assoc., **20**, 2: 80-88. June, 1949.

The author presents a modification of a previous ring test (J.S.A.V.M.A., 19, 2: 130-134. 1948) used for detecting brucellosis-infected milk. The modified test can be read on individual cows and gives a high percentage of accuracy.

The new test not only has the advantage of use on individual cows but can be used to determine whether an animal is responding to the test due to infection reactions or resulting from vaccinal reaction. This is done by degree of color reaction when the antigen is added.

K. M. Dunn

**261. Aureomycin therapy in human brucellosis due to *Brucella abortus*.** A. I. BRAUDE, W. H. HALL and W. W. SPINK, Univ. of Minnesota, Minneapolis. J. Amer. Med. Assoc., **141**, 12: 831. Nov. 19, 1949.

The treatment of 16 patients from whose blood *Br. abortus* was isolated by culture is described. All cases required hospitalization. Seven patients had shown symptoms for less than 2 mo. and were considered to have acute infections; symptoms had been present in the remaining patients for more than 4 mo. and these were considered chronic. Ages ranged from 18 mo. to 60 yr; 14 patients were male and 2 were female. In 6 patients there were either other diseases or complications due to brucellosis. Arthritis of the hip was present in 2 boys. The 18-mo. old infant suffered severe anemia. Seven patients had been treated previously with other drugs, presumably unsuccessfully. Dosages and results are described for each patient and these and other data are presented in tabular form.

Rapid and striking improvement occurred in all cases. There was bacteremia after treatment in only 1 patient and clinical relapse in 2 others. Aureomycin is believed to be superior to other drugs in the treatment of brucellosis.

D. P. Glick

**262. Infectious or epizootic infertility of cattle.** M. W. HENNING, Inst. of Onderstepoort, Pretoria, S. Africa. J. So. African Vet. Med. Assoc., **20**, 1: 9-15. March, 1949.

This is an insidious, infectious disease of the genital organs of the bovine. The female will show a thick, tenacious, muco-purulent discharge from the vagina and in the male there is fibrosis and induration of the epididymis and testis. The etiological agent is not known. However, it is believed to be one of the most serious diseases of breeding stock. The disease can be spread by coitus only. The incubation period is a matter of a few days in the cow, but clinical evidence in the bull may not be manifested for 4 to 10 wk.

There is no sure treatment for this disease.

The cow may fail to show active symptoms after a period of time but it is felt that she is still an active transmitter of the disease. Various antiseptics are being used in irrigating the vagina and uterus. Lugol's iodine,  $\text{KMnO}_4$  and flavine preparations have been of some value for treatment. None of these have given 100% recovery. The use of artificial insemination seems to be the best method of preventing a spread of the disease.

K. M. Dunn

**263. Foot and mouth disease problems in southern Africa.** GILLES, De Rock Inst. of Onderstepoort, Pretoria, S. Africa. J. So. African Vet. Med. Assoc., 20, 1: 1-8. March, 1949.

The first outbreak of foot and mouth disease in southern Africa occurred in 1931. Since that time there has been a gradual spread of the disease throughout the area. Several measures have been undertaken to combat the disease. Some areas have resorted to the vaccination of all infected and contact herds with virulent virus. These workers obtain 99% infection and in a short period of time all infection was eliminated from the herds naturally and artificially infected. However, the use of the virulent virus will aid in the spread of the disease to non-immune cattle. Other areas have set up a program of isolation of infected cattle by fencing in areas where outbreaks have occurred. This method has not had a great deal of success. Still another program, which has given by far the best success, is the "slaughter-out policy." The author points out that this latter program should be followed wherever possible and if this cannot be accomplished, a program of vaccination with either a vaccine prepared according to the method of Schmidt-Waldmann or the crystal violet vaccine. Vaccination with a virulent virus should not be used.

Several different strains of foot and mouth disease virus have been isolated in various infected areas. This has added to the general confusion of the possible original source of infection for the southern African area.

K. M. Dunn

Also see abs. no. 253.

## CHEESE

### A. C. DAHLBERG, SECTION EDITOR

**264. Improved sweet curd cottage cheese.** N. G. ANGEVINE, Meyer-Blanke Co., St. Louis, Mo. Milk Plant Monthly, 39, 1: 58-60, 67. Jan., 1950.

The manufacture of sweet curd cottage cheese by the short method involved pasteurization of fresh good quality skim milk at  $143.5^\circ\text{F}$ . for 30 min. or  $161^\circ\text{F}$ . for 15-19 sec. Following pasteurization, the temperature is reduced to  $90^\circ\text{F}$ .

and 5-6% of fresh active starter added. In about 1-1.5 hr. the proper amount of coagulator may be added with proper but not prolonged agitation. Then as soon as the curd is sufficiently firm, usually 3.5 hr. after setting and a whey acidity of 0.50-0.52%, the curd should be cut into even sized 0.5-0.625 in. cubes. Cooking during the first 15 min. should be at a temperature of  $110^\circ\text{F}$ . The average cooking time will vary from 50 min. to 1.5 hr., and involves a final temperature of  $120^\circ\text{F}$ . The whey then may be drained and the curd washed with tap water at a temperature of  $85^\circ\text{F}$ . The second washing should be at  $50-60^\circ\text{F}$ . Following drainage, the curd may be creamed in the vat by adding 40 lb. of 14% dressing to each 100 lb. of curd and salted at the rate of 2 oz./gal. of dressing. J. A. Meiser, Jr.

**265. Manufacture of American type cheese.** J. B. STINE. (Assignor to Kraft Foods Co.) U. S. Patent 2,494,638. 3 claims. Jan. 17, 1950. Official Gaz. U. S. Pat. Office, 630, 3: 710. 1950.

American cheese is made by setting, cutting and cooking the curd in the conventional manner, draining off a portion of the whey, adding salt to the curd suspended in the remaining whey, cooking further, then draining the whey after forming the curd into blocks, and finally curing in the usual way.

R. Whitaker

**266. Method of pressing cheese.** R. MIOLLIS. U. S. Patent 2,492,878. 7 claims. Dec. 27, 1949. Official Gaz. U. S. Pat. Office, 629, 2: 1138. 1949.

This process provides for a method of removing whey by pressing cheese in forms in multiple vertical columns.

R. Whitaker

**267. Cheese manufacture.** J. B. STINE. (Assignor to Kraft Foods Co.) U. S. Patent 2,494,637. 1 claim. Jan. 17, 1950. Official Gaz. U. S. Pat. Office, 630, 3: 710. 1950.

Cheese curd of the Swiss type when still submerged in the whey in the vat is packed into a mold and formed into the desired shape. The whey is then drained off, the curd allowed to mat and knit, after which it is cured in the usual way.

R. Whitaker

**268. Emmenthaler cheese.** J. B. STINE. (Assignor to Kraft Foods Co.) U. S. Patent 2,494,636. 4 claims. Jan. 17, 1950. Official Gaz. U. S. Pat. Office, 630, 3: 710. 1950.

Swiss cheese is sealed in a flexible, elastic moisture proof bag, placed in a mold and cured.

R. Whitaker

**269. Beitrag zur Käsereitauglichkeit von Silo-**

**milch und Bakteriologie des Tilsiter Käses.** (A contribution to the fitness of ensilage milk for cheese making and the bacteriology of Tilsit cheese.) (English Summary.) K. J. DEMETER, A. JANOSCHEK and A. RAU. *Die Milchwissenschaft*, 4, 1: 3-14. Jan., 1949.

Comparison trials were made on the making and ripening of tilsit cheese from lots of raw milk obtained from selected groups of cows fed on different rations. One group was fed a normal ration consisting primarily of alfalfa hay, grains and straw, whereas the other group was fed a silage ration consisting primarily of alfalfa hay, silage (clover grass silage, corn silage) and straw.

Results of 69 trials showed that curd formation was somewhat slower with milk from cows on silage ration than with milk from cows on normal ration. This was due chiefly to the lower initial acidity of the milk and the slower action of rennet upon the milk from cows on silage ration. The ripened cheese made from the latter milk frequently was criticized for having a sweetish putrid taste and was inferior to the cheese made with milk from cows on normal ration.

Bacteriological studies did not show any pronounced difference in the microbial flora of the cheese made from the 2 types of milk.

I. Peters

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**270. Formulas for the commercial use of whey in bakery goods.** L. V. ROGERS, Bureau of Dairy Industry, USDA. BDIM-Inf.-89. (Mimeoprint) Jan., 1950.

Whey incorporation in bakery goods results in more tender products which remain soft and of good eating quality longer. The advantages of different whey products are discussed. Formulae for white whey-bread, rich yellow layer whey-cake, cocoa whey-cookies, oatmeal coconut whey-cookies, yeast raised whey-doughnuts and whey sweet-dough are given, along with directions for the making of each product.

F. E. Nelson

**271. Confection stock.** M. P. ANNARILLI. U. S. Patent 2,495,217. 16 claims. Jan. 24, 1950. Official Gaz. U. S. Pat. Office, 630, 4: 945. 1950.

A candy base is described containing, in addition to sugar, cocoa, cream of tartar and flavoring, a combination of powdered cream, whole milk, skim milk and roasted milk. R. Whitaker

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**272. The problem of coliform bacteria in mar-**

**ket milk.** K. G. WECKEL, Univ. of Wisc., Madison. *Milk Plant Monthly*, 39, 1: 16-18, 20. Jan., 1950.

The source and significance of coliform organisms in raw and pasteurized milk are discussed. Methods of detecting these organisms and interpretation of these tests are presented. Supervisory measures necessary for controlling contamination of dairy products are (a) preventing contamination of the pasteurized product with raw milk or extraneous matter, (b) adequate and complete pasteurization of milk products and (c) thorough sterilization of processing equipment.

J. A. Meiser, Jr.

**273. The problem of thermophilic and thermoduric bacteria in milk.** R. N. DOETSCH, Univ. of Maryland, College Park. *Milk Plant Monthly*, 38, 12: 32, 33, 36. Dec., 1949.

Pasteurization at either 143° F. for 30 min. or 161° F. for 16 sec. supposedly reduces the bacteria count of milk 90-99%. When the expected "kill" is not obtained, it is due to the growth of thermophilic bacteria or the survival of thermoduric organisms. Thermophilics, having an optimum growth temperature of 55-70° C., seldom are a problem until the milk arrives at the dairy plant. Preheating and pasteurization equipment, if used for several hours, favors the growth of these organisms, especially if residual milk solids accumulate. Their presence can be detected by the agar plate or oval tube method, using a 55° C. incubation temperature, or the Breed method. Thermoduric, unlike thermophilic, organisms usually are introduced into the milk at the farm in large numbers from improperly cleaned and sterilized equipment. Once in the plant they seed the equipment and become a problem. Although detectable by agar plate incubation at 35° C., they must be controlled by proper sanitation techniques in the plant and on the dairy farm.

J. A. Meiser, Jr.

**274. Hexadecenoic acid as a growth factor for lactic acid bacteria.** J. B. HASSINEN, G. T. DURBIN and F. W. BERNHART. Wyeth, Inc., Mason, Mich. *Arch. Biochem.*, 25, 1: 91-96. Jan., 1950.

Employing the usual acidimetric procedures for microbiological assays, the growth-promoting properties of oleic acid (9-octadecenoic acid) and palmitoleic acid (9-hexadecenoic acid) were compared for *Lactobacillus arabinosus* 17-5 (ATCC) and a mutant strain of *L. bifidus*. At low levels (10-20  $\gamma$ /ml.) hexadecenoic acid accelerated the growth of *L. bifidus*, but slightly higher levels (30  $\gamma$ /ml.) depressed its growth entirely. It was demonstrated that the inhibition due to high



levels of hexadecenoic acid was removed by either palmitic acid or stearic acid and most effectively by a mixture of these 2 acids. Both low and high levels of oleic acid (as much as 200  $\gamma$ /ml.) supported good acid production, and the synergistic action of oleic acid and a mixture of palmitic and stearic acid was slight. In the absence of biotin, *L. arabinosus* responded similarly to oleic acid and hexadecenoic acid; with the latter levels up to 100  $\gamma$ /ml. were not toxic. Decreased acid production occurred when a mixture of palmitic, stearic and oleic acids was included in the medium; however, a mixture of hexadecenoic, palmitic and stearic acids greatly stimulated the growth of *L. arabinosus*. H. J. Peppler

**275. Neue Züchtungsverfahren für *Penicillium camemberti*.** (New methods of culturing *Penicillium camemberti*.) (English Summary.) W. KUNDRAT. *Die Milchwissenschaft*, 4, 1: 23-24. Jan., 1949.

Cultures of *Penicillium camemberti* grown on sterilized potato cubes (7-10 mm.<sup>3</sup>) produced twice as many conidiospores as cultures grown on parchment moistened with sterile whey. However, mold contaminants can be detected, more easily on the parchment layer and the parchment with the mold on it can be dried, packaged in sterile containers and shipped more economically than if the mold is grown on potato cubes.

I. Peters

Also see abs. no. 254, 257, 258.

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**276. Colorimetric determination of reducing sugars with triphenyltetrazolium chloride.** A. M. MATTSON and C. O. JENSEN, Pa. State College, State College. *Anal. Chem.*, 22, 1: 182-185. 1950.

An aqueous solution of triphenyltetrazolium chloride is colorless, but it forms a red, water-insoluble compound, triphenylformazan, when it is reduced by reducing sugars. The quantity of formazan formed is proportional to the quantity of reducing sugars present. By controlling time, temperature and alkalinity, the reaction can be used as the basis of a colorimetric method for the determination of reducing sugars. The method is applied to the determination of lactose in milk and of glucose and fructose in honey.

B. H. Webb

**277. Kinetics of the enzyme-catalyzed oxidation of lactic acid.** I. M. SOSQUET and K. J. LADLER. Catholic Univ. of America, Washington, D. C. *Arch. Biochem.*, 25, 1: 171-184. Jan., 1950.

Lactic dehydrogenase extracted from calves' hearts was employed in a detailed study of the reaction, lactic acid + coenzyme I  $\rightarrow$  pyruvic acid + reduced coenzyme I. The rate of reaction was observed to reach a constant limiting value with respect to lactic acid concentration, but reaches a maximum and then diminishes with respect to coenzyme concentration. The data obtained with this system suggest that the coenzyme can be absorbed on its own type of site as well as on the lactic acid type of site. The lactic molecule, however, can be adsorbed only on one type of site. Marked increases in activation energy with increasing concentrations of lactate and coenzyme provide evidence that binary and ternary complexes (apoenzyme-lactate-coenzyme) are formed exothermically and with an entropy decrease; the ternary complex being more stable than the binary complex by 4 kcal. In contrast with this behavior of hydrogenases, the process of complex formation for reactions catalyzed by urease and pepsin is reported to proceed endothermically and is accompanied by an increase in entropy. H. J. Peppler

**278. Studies of the nutritive impairment of proteins heated with carbohydrates. II. In vitro digestion studies.** J. R. LOWRY and R. THIESSEN, JR., General Foods Corp., Hoboken, N. J. *Arch. Biochem.*, 25, 1: 148-156. Jan., 1950.

Protein-dextrose complexes prepared by autoclaving equal weights of moist casein or wheat gluten with dextrose were digested *in vitro* by pepsin, chymotrypsin and pancreatin but were resistant to trypsin and papain. Amino groups resembling lysine and arginine, in the side chain of the enzyme substrate, were found to be vital to the action of trypsin. Lysine appeared to be especially important because some of its amino groups are free to combine with reducing sugars. When zein, a protein low in lysine, is autoclaved with dextrose, the light-colored complex is readily digested by trypsin. Since papain attacks linkages similar to those split by trypsin, the reaction of dextrose with free amino groups in the side chains also produces a protein complex resistant to digestion. When the enzymes are employed in physiological sequence at the proper pH, the prior digestion with pepsin does not alter the activity of trypsin. If the *in vitro* blocking of tryptic action reported here also occurs *in vivo*, the failure of the casein-dextrose complex to support the growth of young rats would be explained. H. J. Peppler

**279. The "browning" reaction of proteins with glucose.** A. MOHAMMAD, H. FRAENKEL-CONRAT and H. S. OLCOTT. Western Regional Research

Laboratory, Albany, Cal. Arch. Biochem., **24**, 1: 157-178. Nov., 1949.

A detailed investigation was made to determine the effects of temperature, pH, and other variables on the rate of browning, the properties of such "browned" protein derivatives and the protein groups involved in the reaction of crystalline bovine serum albumin (BSA) in buffered solutions with glucose. The typical procedure used to measure the development of color employed 1 g. of protein dissolved in a mixture of 2 ml. 3.4M  $K_2HPO_4$  buffer (pH 7.6) and 10 ml. 37.5% glucose solution. After the reaction period, samples of 1 ml. were diluted to 10 ml. for transmission data in a spectrophotometer at 500 m $\mu$ .

The rate of the browning reaction is proportional to the temperature and pH. In the temperature range of 25-65° C. the same reaction mechanism occurs. A short induction period is followed by a protracted period during which the increase in color is linear with time. Increases in alkalinity accelerate the browning reaction, suggesting hydroxyl-ion catalysis. The development of browning was essentially the same when the buffer was phosphate, carbonate or veronal.

Traces of Cu accelerated the rate of browning of BSA in glucose solution, but it did not appear to affect the disappearance of amino groups.

Derivatives isolated after the reaction of BSA and glucose revealed similar properties. The BSA-glucose product was insoluble at its isoelectric region (pH 4.2-4.4) and resisted coagulation by heat in solutions heated at 100° C. for several hours, both below (pH 3.5) and above (pH 7.0) the isoelectric region. Details of electrophoretic homogeneity, osmotic pressure measurements, ultracentrifuge pattern, amino acid analyses and relative rates of trypsin digestion of BSA and its glucose derivative are discussed.

The primary site of the browning reaction is at the free amino groups, which decrease as the reaction proceeds. Masking them, as by acetylation, prevents browning at 53° C. or lower. At 70° C. secondary reactions, possibly due to guanidyl and indole groups, are involved.

From the observations that the browning of proteins proceeds more readily with acetaldehyde and propionaldehyde than with glucose, it is apparent that the aldehyde group is essential.

H. J. Peppler

**280. X-ray diffraction analysis of vaccenic acid.** J. H. BENEDICT and B. F. DAUBERT. Univ. of Pittsburgh. J. Am. Chem. Soc., **71**, 12: 4113-4. Dec., 1949.

A comparison of X-ray data obtained for natural vaccenic acid and synthetic vaccenic acid with those obtained for elaidic acid reveals that syn-

thetic vaccenic acid and elaidic acid possess similar crystal structures. Dissimilarities observed in the diffraction patterns were attributed chiefly to differences in the angles of tilt in the crystals; however, the possibility of structural differences could not be eliminated. Vaccenic acid obtained as a product of the hydrogenation of  $\beta$ -elacostearic acid exhibited a pattern similar to that obtained with the natural vaccenic acid. H. J. Peppler

**281. Mikrobiologische Bestimmung von Wuchsstoffen in Normal- und Silage Milch. (Microbiological determinations of growth factors in normal and in ensilage milk.)** (English summary.) E. F. MÖLLER. Die Milchwissenschaft, **4**, 1: 14-18. Jan., 1949.

Microbiological assays were made on milk dialysates for the determination of thiamine, pantothenic acid, nicotinic acid and p-amino benzoic acid with *Streptobacterium plantarum* and *Proteus vulgaris* as test cultures. Results of trials conducted over a 2-yr. period using milk from cows fed on a silage ration or on a normal ration showed that both milks had a similarly high concentration of the above growth factors, which concentrations were sufficiently high to permit proper growth of *Streptococcus lactis* in the milk. Apparently, however, silage milk was lower in an unknown factor I, required for the growth of *S. plantarum*.

The author believes failure to manufacture hard cheese from silage milk cannot be attributed to the lack of growth factors in such milk.

I. Peters

**282. Ultracentrifugal study of bovine plasma protein fractions.** V. L. KOENIG and K. O. PEDERSEN. Univ. Upsala, Sweden. Arch. Biochem., **25**, 1: 97-108. Jan., 1950.

Ultracentrifugal studies on the bovine plasma protein fractions resulting from the application of low temperature alcoholic fractionation procedures developed by E. J. Cohn and his associates were made with the Svedberg oil turbine velocity centrifuge operating at 59,000 r.p.m. The sedimentation diagram for Fraction I (fibrinogen) indicates a good preparation of the protein, having only a small amount of a heavier component. The sedimentation diagram of Fraction II ( $\gamma$ -globulin) gave evidence of about 18% of a heavier component, while that of Fraction III-1 ( $\beta$ -globulin) reveals the presence of at least 2 heavier components amounting to about 13% and a very small quantity of a lighter component. Fraction IV ( $\alpha$ -globulin and other components) presents a complex mixture of proteins consisting of 3 main components. Crystalline albumin, considered one of the purest of the

plasma proteins isolated, gave evidence of small quantities of nondescript heavier components and a very small amount of lighter material. The values for the sedimentation constants extrapolated to zero concentration and zero  $n$ , the refractive index increment, were found to be as follows: Fraction I, 8.43-8.62S; Fraction II, 7.28-7.31S; Fraction III-1, 7.37S; Fraction IV, 19.45-19.68S, 7.31-7.46S, and 5.64-5.76S; crystalline albumin, 4.73S. H. J. Peppler

**283. Some characteristics of mare's colostrum and milk.** A. D. HOLMES and H. G. LINDQUIST, Mass. State Coll. J. Am. Diet. Assoc., **23**, 11: 957-961. Nov., 1947.

Determinations were made of pH, fat, total solids and reduced ascorbic acid for 23 d. of milk from 3 Percheron mares, and for 15 d. of milk from 1 Palomino mare. The pH value of the colostrum was very stable for the first 4 d; on the 5th d. it was decidedly higher and from the 5th to the 21st d. it was quite constant. The averaged fat for the 1st 4 d. of lactation was 2.5%. The fat decreased slowly from the 5th d. to the end of the experimental period. The reduced ascorbic acid was relatively low in the colostrum but increased fairly steadily from the 1st to the 16th d. of lactation and then decreased slightly. At the 1st estrual period, about 9 d. postpartum, both the fat and ascorbic acid content changed from the values before or after the estrual period.

R. N. Davis

Also see abs. no. 309.

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**284. Method and apparatus for heating milk.** LE R. R. HAWK. (Assignor to Golden State Co.) U. S. Patent 2,492,635. 6 claims. Dec. 27, 1949. Official Gaz. U. S. Pat. Office, **629**, 4: 1075. 1949.

Milk or other liquid is heated rapidly in this device which consists of a perforated blade revolving in a housing, similar in design to a simple centrifugal pump. Steam is fed into the housing where it is mixed intimately with the milk by the revolving blade. The heated milk is discharged continuously through an opening in the periphery of the housing. R. Whitaker

**285. Process for heat treating milk and cream in containers.** J. O. FOWLER. U. S. Patent 2,493,663. 2 claims. Jan. 3, 1950. Official Gaz. U. S. Pat. Office, **630**, 1: 264. 1950.

To prevent breakage of bottles of hot milk and cream following in-bottle pasteurization, the bottles are held for 2-4 min. in a moisture-free, still

atmosphere at 40-50° F. to cool the bottles through the cooling effect of evaporating the film of water on the outside surface. Following this step, the bottles may be submerged in chilled water to complete cooling. R. Whitaker

**286. Process for heat treating milk and cream.** J. O. FOWLER. U. S. Patent 2,493,664. 3 claims. Jan. 3, 1950. Official Gaz. U. S. Pat. Office, **630**, 1: 264. 1950.

An in-bottle pasteurizing system for milk and cream is described. The bottles are placed in racks and heated and cooled by moving the racks through a succession of tanks of hot and cold water. R. Whitaker

**287. Pumps for pure liquids.** F. A. KRISTAL. Operating Engr., **3**, 1: 38-39. Jan., 1950.

Pumps for handling foods must be resistant to corrosion and easy to take apart for cleaning. They must not churn the liquid. Some pumps must handle solids such as vegetables in soups without injuring the solids. All food pumps must be designed so that food will not be tainted or contact lubricants.

The sanitary pumps designed for food products are the centrifugal with flat-blade impeller, centrifugal with 3 curved blades, internal gear, rotary and reciprocating. Photographs and drawings are offered to aid in explaining the pumps.

H. L. Mitten, Jr.

**288. How much torque is needed to start centrifugal pumps?** R. CARTER, Worthington Pump & Machinery Corp., Harrison, N. J. Power, **94**, 1: 88-90. Jan., 1950.

Proper selection of a power unit for a centrifugal pump seldom causes worry, for centrifugal pumps are relatively easy to start. Time needed for starting and bringing the load to speed depends upon the margin of torque available to accelerate the load and the flywheel effect of rotating parts. Centrifugal pumps have low flywheel effect and low-starting torque requirements, so high-starting torque motors are not required. Curves are presented to show characteristics of typical centrifugal pumps. H. L. Mitten, Jr.

**289. BTU are good to last drop.** A. W. SHEPARD, San Diego, Calif. Operating Engr., **3**, 1: 28-29. Jan., 1950.

When a boiler is taken out of service it must be cooled down. Where there is not a great reserve capacity it may be advantageous to conserve as much of the heat as possible rather than to waste the heat in the normal cooling procedures. Where the boiler has a superheater with drains to

the blowdown tank and a low-pressure exhaust system used for a secondary purpose, connections can be made to connect the high and low pressure systems. The purpose of the connections is to take the heat from the high pressure side in small usable amounts and rise it in the low pressure system. When the boiler is to be taken out of service the fans are shut off and the boiler doors are left closed. The valve connecting the systems is then opened slowly. This permits even cooling of all boiler parts at a rate which prevents harmful stresses. Proper relief valve and safety devices should be provided.

H. L. Mitten, Jr.

**290. Winter tips on cooling towers.** H. E. DEGLER. *Operating Engr.*, 3, 1: 36-37. Jan., 1950.

To avoid difficulties follow the manufacturer's operating instructions. Fog is usually worse during mild winter weather of 50-60° F. Fog is airborne droplets formed by vapor condensation. Reduce fog trouble by placing tower away from railroad tracks and highways, maintain low air temperatures at tower outlet, put as much air through tower as conditions permit and keep to minimum the ice formation on towers. Icing may be prevented during cold weather by keeping the tower temperatures as high as practicable by reducing fan speeds, reducing water flow and by passing water to part of tower.

H. L. Mitten, Jr.

**291. New water-hardness test is faster and gives more accurate results.** J. M. MARCY, Hall Laboratories, Inc., Pittsburgh, Pa. *Power*, 94, 1: 105-108. Jan., 1950.

The test is sensitive to small amounts of water hardness, 1ppm. of hardness as  $\text{CaCO}_3$  being detectable. There is no limitation on sample size, since no scum or salt effect obscures the endpoint. The test is rapid and is applicable to high or low hardness and may be used in brines. Reagents consist of a buffer containing  $\text{NH}_4\text{OH}$  and  $\text{NH}_4\text{Cl}$  together with small amounts of magnesium and disodium salt of ethylene-diamine-tetra-acetic acid; sulphide solution containing  $\text{Na}_2\text{S}$  and  $\text{NaOH}$ ; an indicator solution of adjusted chrome-black T in methyl alcohol; and a titrating solution containing complex-forming disodium salt of ethylene-diamine-tetra-acetic acid.

In making the determination, the water sample is buffered at a pH of 10 and, if necessary, sulphide solution is added. The indicator is added and the sample is titrated with the disodium salt of ethylene-diamine-tetra-acetic acid. The end point occurs when the last trace of reddish tinge disappears.

H. L. Mitten, Jr.

**292. Simplifying plant lubrication.** J. G.

O'NEILL, JR., Sinclair Refining Co., New York City. *Power*, 94, 1: 86-87. Jan., 1950.

Recommendations in instruction books and on machine name plates are generally confusing. Some manufacturers list oil by brand name, some by viscosity at various temperatures, others by SAE (Society of Automotive Engineers) grade or AGMA (American Gear Manufacturers Association) number. The plant operator in his attempt to follow the manufacturers' instructions soon has many different lubricants on hand and has invested considerable in dispensing equipment for each product.

In an effort to simplify the lubrication problem, leading machinery manufacturers were consulted and mechanical units were classified. The type and viscosity range of the required oils were listed. This showed that many machines could use the same oils. In applying this information, 1 plant using 376 different lubricants reduced the number to 15. Another using 99 found it possible to get along with 9.

A chart listing the Saybot Universal Viscosity, SAE grade and the AGMA number along with a classification of applications is presented so the reader can easily convert any unit of viscosity to any other unit and compare the applications of the various oils.

H. L. Mitten, Jr.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**293. Controlling wastage in milk plants.** H. HELMBOIT. Sheffield Farms, Springdale, Conn. *Milk Plant Monthly*, 38, 11: 34-35, 53. Nov., 1949.

Wastage in milk plants is not confined to the product alone but occurs in supplies as well. Keeping these latter losses to a minimum involves (a) accurate inventory records so organized as to show where the losses occur, (b) teaching plant personnel the importance of thrift, (c) foresight as to more efficient methods of plant operation and (d) perseverance. Reducing wastage is not an expensive program but does require constant thought and supervision.

J. A. Meiser

**294. Better accounting for milk plants.** FRED MERISH. *Milk Plant Monthly*, 38, 12: 54-57. Dec., 1949.

Many business failures are due to faulty accounting practices. For maximum efficiency and maximum profit plant operators must keep adequate and accurate records. In general, 4 classes of records must be kept: (a) financial records, (b) production records, (c) auxiliary records and

(d) business statements. If these accounts are kept systematically, fewer business failures should result.

J. A. Meiser, Jr.

**295. Better business management for milk plants.** FRED MERISH. *Milk Plant Monthly*, 39, 1: 34, 36, 50. Jan., 1950.

Business management is a matter of budgeting, cost control, sales control, business analysis and competent financial supervision. This provides the milk plant operator with a clear perspective of his own operations and increases his chances of survival in a competitive market.

J. A. Meiser, Jr.

**296. The dairy sales manager's duties.** C. H. BEHLE, Breuningers Dairies, Philadelphia, Pa. *Milk Plant Monthly*, 38, 12: 50, 52. Dec., 1949.

A sales manager's duties are leading, advising and aiding sales personnel. Rather than force personal decisions on supervisors and foreman, it is advisable to solicit their opinions which should be weighed carefully before reaching decisions. Advance information gained by these group conferences enables personnel to promote sales policies with more enthusiasm, thus insuring better sales returns.

J. A. Meiser, Jr.

**297. Sales training for routemen pays off.** F. FLAGG. *Milk Plant Monthly*, 38, 11: 77-78. Nov., 1949.

Immediately following a sales training course for sales personnel, a survey of a New Haven, Conn. marketing area showed that a considerable amount of new business could be solicited by enterprising routemen. Allocating 100 qt. of new business/routeman, each driver was given 3 mo. in which to pick up the added business. As an added incentive, routemen received a bonus of \$2.00 for each new customer maintained for a 4-mo. period.

J. A. Meiser, Jr.

**298. New customer campaign spurs collections and reduces bottle losses.** P. L. ANDERS. *Milk Plant Monthly*, 38, 11: 71-72. Nov., 1949.

According to this plan, routemen receive \$4.00 for each new customer only if their bottle losses did not exceed 3% of their daily load and if their outstanding bills did not increase. The resulting 70% drop in bottle losses and a reduction of one-half in outstanding bills, indicated that the \$4.00 bonus was effective.

J. A. Meiser, Jr.

**299. Contest boosts sales of chocolate milk.** Anonymous. *Milk Plant Monthly*, 38, 11: 62-63. Nov., 1949.

To increase the sales of chocolate milk, an old-fashioned sales contest was promoted; however, in conjunction with added commissions to the participants, prizes were given to the wives of the men who had obtained the greatest increase in sales during a 4-wk. period. With the added incentive from wives, the contest was highly successful.

J. A. Meiser, Jr.

**300. Incentives spur sales and collections.** R. MILLER. *Milk Plant Monthly*, 38, 11: 74-75. Nov., 1949.

In order to establish new delivery routes, customers were taken from established routes and used to form the nucleus of a new route. Regardless of the number of points taken away, the company guaranteed the routeman his previous month's earnings for the next 2 mo. This gave the driver additional time in which to rebuild his route and permitted him to transfer customers that could be better served by other routemen. Although the personnel were paid according to sales volume, a delivery of 350-400 qt. was considered to be an adequate day's work, in that it allowed the routemen sufficient time to solicit business, meanwhile assuring them their regular or even increased earnings.

J. A. Meiser, Jr.

**301. Self service stores.** R. L. STEPHENS. High's Dairy Products Co., Washington, D. C. *Ice Cream Trade J.*, 46, 1: 24, 60. Jan., 1950.

After a pilot store proved successful, 10 counter service retail stores were converted to the self-service type. All stores are of the "dry stop" variety with 95% of the sales being carry out sales.

Self-service was installed as a means of reducing the waiting time for customers making it possible to serve more customers and to increase the sale of packaged ice cream. The self-service stores require a larger investment, higher power bills and some customers require help; however, these disadvantages are far outweighed by other advantages. Sales of all items increased in the self-service stores, customers were happier because of faster service, employees were happier because of less work and packaged sale was increased, now outselling hand packed (3-1). Sales on the 0.5 gal. bulk family package increased, also.

A hand dipping department for cones and pints is essential but little extra help is needed for it. A standard layout for stores was developed with minimum measurements of 14×50 ft. An exhaust fan was necessary for proper ventilation. If possible, all compressors should be located in the basement. Store equipment investment and electrical bills are about 3 times that of the counter type store. Self service push carts are not used by customers. Small items should be lo-

cated at the front of the store to prevent pilfering. Some customers need help in locating items. All collections are made at check out counters and skeptical customers could be taught by salesladies that ice cream and milk kept in the open cabinets were in good condition.

W. H. Martin

**302. Drug store study.** H. H. ROBBINS, Parafined Carton Research Council, Ice Cream Trade J., **45**, 12: 32, 34, 84. Dec., 1949; *ibid.*, **46**, 1: 26-28, 72. Jan., 1950.

Eight independent drug stores located in the neighborhood shopping areas in Los Angeles, Birmingham, Buffalo, Milwaukee, Philadelphia, St. Louis, Wichita and Springfield, Ohio, with average annual sales of \$103,302/store were surveyed. The fountain alone accounted for 39.2% of the stores' sales transactions and tobacco, candy and magazines accounted for 31.7%. Seven out of 10 sales were made by these 2 departments. The fountains gross profit was 39.9% of sales compared to 53.8% for prescriptions. The average fountains net profit was 1.7%, with 4 stores showing a net of 5.2-17.5% and 4 stores showing a loss of 0.9-14.9%. The 4 profitable fountains averaged 12.4%, while 4 unprofitable ones showed a net loss of 9.1¢ for every dollar sale. At the fountain, factory packed ice cream showed a gross profit of 37.6%, hand packed 33.2% and novelties 28.5%.

Factory packaged ice cream was the only product handled profitably in all test fountains. The rate of turnover was almost 3 times as great as that for hand packed ice cream which was the second most profitable product in the soda fountain. Factors in favor of packaged ice cream are that it needs no training to sell, low labor, rent and inventory costs, high turnover and uniformity of product.

W. H. Martin

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

**303. The examination of bull semen.** S. W. J. VAN RENSBURG and N. C. STARKE, Inst. of Onderstepoort, Pretoria, S. Africa. J. So. African Vet. Med. Assoc., **20**, 2: 70-79. June, 1949.

This is an outline of the procedure to follow in the collection and evaluation of bull semen. The author lists the following tests which should be run on semen: physical properties, motility, density, pH, methylene blue reduction test and morphology. Each test is discussed in detail as to various abnormal conditions which may be detected. A very good diagram of different types of abnormal sperm is presented.

K. M. Dunn

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**304. Stock watering trough.** L. E. MEISNER. U. S. Patent 2,490,824. 1 claim. Dec. 13, 1949. Official Gaz. U. S. Pat. Office, **629**, 2: 408. 1949.

This outdoor stock watering trough consists of 2 troughs, an upper trough for large animals and a lower one for small animals. The device is enclosed in insulated walls. Counter-weighted doors, normally in the closed position, are easily opened by the animal. A small oil lamp and means for circulating warm air is provided for sub-freezing weather.

R. Whitaker

**305. Vacuum line contamination—Its causes and its cures.** I. E. PARKIN, Penn. State College. Milk Plant Monthly, **38**, 12: 68-69. Dec., 1949.

The operating efficiency of a milking machine is greatly reduced by partially clogged vacuum lines, swollen and porous stanchion hoses and loose drive belts. Preventing dust, bedding and other foreign materials from entering the system and periodic flushing of the vacuum lines with a lye solution and successive hot water rinses will improve the efficiency of the machine and also provide better quality milk.

J. A. Meiser, Jr.

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**306. Emulsifiers and their role in ice cream.** H. L. CASLER, Germantown Manufacturing Co., Philadelphia 3., Pa. Ice Cream Trade J., **42**, 12: 48, 77. Dec., 1949.

Emulsifiers classed as "esters" are combinations of long-chain fatty acids such as stearic, palmitic or oleic with one of the higher alcohols, such as glycerol or sorbitol. The esters differ from the fats in that not all the possible linkages of the alcohol are taken up by the fatty acid, thereby producing a compound which has affinity for both fat and water.

Each molecule of the ester may be thought of as a rod, the fatty acid end is soluble in fat and the alcohol end is soluble in water, acting as a link preventing separation. Esters also are powerful surface active agents which move to any interface where fat and water meet and greatly reduce the interfacial tension, which is the force which pulls like globules together to form the largest possible masses. With this force removed, the homogenizer easily can reduce the butterfat to submicroscopic globules of less than 1-10,000th the diameter of a pin. These esters spread over all interfaces, entirely surrounding the tiny fat globules, forming protective films which in con-

junction with the stabilizer prevent the globules from clumping.

Emulsifiers have been in use in the margarine and shortening industry and by bakers for years. They are non-toxic and have about the same nutritional value as true fats. W. H. Martin

**307. Polyoxyethylene esters for improving frozen confections.** A. B. STEINER and A. MILLER. (Assignors to Kelco Co.) U. S. Patent 2,493,324. 12 claims. Jan. 3, 1950. Official Gaz. U. S. Pat. Office, **630**, 1: 176. 1950.

An ice cream improver consisting of a hydrophilic colloid and a polyoxyethylene ester of stearic acid as an emulsifying agent is described.

R. Whitaker

**308. Storing cream for use in ice cream.** J. W. STULL, Univ. of Arizona, Tucson. Ice Cream Trade J., **46**, 1: 46, 86. Jan., 1950.

Cream for storage should not come in contact with Cu or Fe during processing and should be separated from high quality milk. A product containing either 40 or 80% fat may be stored. In the case of 40% cream, 10% sugar may be added before pasteurization to prevent destabilization during freezing. The pasteurization temperature should be 170° F. for 15 min. and the cream cooled to 40° F. and stored at -10-20° F.

Antioxidants, such as nordihydroguaiaretic acid (NDGA) in concentrations as low as 0.005% of the fat content of the cream, may be used to prevent oxidation. When NDGA is used, pasteurization temperature may be reduced to 150° F. for 30 min. The frozen product may be thawed by placing in a 40° F. room or it may be crushed in a sanitary type ice crusher and incorporated directly into the mix.

W. H. Martin

**309. Detecting foreign fats in ice cream.** W. H. MARTIN, W. D. RUTZ and C. H. WHITNAH, Kansas State College, Manhattan. Ice Cream Trade J., **45**, 12: 40, 78. Dec., 1949.

Reichert-Meissl numbers of butter fat used in 4 ice cream mixes averaged 29.24. This value was almost identical with the 29.15 number for fat extracted by the Minnesota reagent churning method from the ice cream. The Polenske number for the control fat was 2.17, compared to 2.19 for the fat extracted from ice cream. The Kirschner number for the control fat was 24.44 compared to 24.63 for the extracted fat.

Determinations were made on fat extracted from 5 experimental samples of ice cream which contained foreign fats. When one-third of the fat was supplied by coconut oil and cottonseed oil, the Reichert-Meissl numbers were reduced from 28.70 to 23.59 and 21.14, respectively. Kirschner

numbers likewise were about one-third lower than the Kirschner number of the butterfat. About 5% adulteration with foreign fats could be detected by the Reichert-Meissl numbers, provided a sample of the butterfat used in the ice cream or a similar sample is available for analysis.

W. H. Martin

**310. Ice cream container.** A. H. BARASCH. U. S. Patent 2,492,832. 5 claims. Dec. 27, 1949. Official Gaz. U. S. Pat. Office, **629**, 4: 1126. 1949.

An edible container for ice cream consisting of 2 parts, bottom and top, is described. The container is expansible to permit maintaining a seal where the top and bottom meet.

R. Whitaker

**311. Ice cream maker and dispenser.** D. A. ELLWELL. U. S. Patent 2,493,395. 3 claims. Jan. 3, 1950. Official Gaz. U. S. Pat. Office, **630**, 1: 195. 1950.

A vertical ice cream freezer having a rotating agitator and a pusher blade, the latter so designed as to eject the finished product through an outlet in the bottom of the cylindrical freezing chamber is described.

R. Whitaker

**312. Method of manufacturing frozen confections.** C. L. and R. and P. BERNARDS. (Assignors to John M. Bernards and Sons, Inc.) U. S. Patent 2,495,403. 3 claims. Jan. 24, 1950. Official Gaz. U. S. Pat. Office, **630**, 4: 991. 1950.

A stick is inserted into a solid core of fruit, covered with ice cream, quick hardened and finally coated in the usual manner to form a frozen novelty on a stick.

R. Whitaker

**313. Soft ice cream.** G. PRINCE, Alexandria Dairy Products Co., Alexandria, Va. Ice Cream Rev., **33**, 7: 50. Feb., 1950.

Freezer fresh ice cream, frozen with a continuous freezer and offered in a variety of flavors will attract customers and increase sales. Soft ice cream combines freshness, flavor appeal and correct serving temperature all in the same product.

Other suggestions offered for increasing the sale of ice cream during the winter months are: (a) Produce higher butterfat (warmer) ice cream in winter, (b) offer a greater variety of flavors, (c) concentrate on the sale of factory filled packaged ice cream with an overrun of from 65-75%, thereby eliminating hand packed ice cream, and (d) provide an insulated bag with sufficient dry ice for the specific holding time necessary for carry-out trade. This service should be

provided at no extra cost to the customer and appropriately may be charged to advertising.

If the consumer can get what he wants and if the product reaches his table at the most favorable serving temperature, summer volume of sales may be approached during the winter months by the ice cream industry, in the opinion of the author.

W. J. Caulfield

**314. Soft ice cream.** P. H. TRACY and D. MOOR, Univ. of Ill., Urbana. *Ice Cream Trade J.*, **46**, 1: 30. Jan., 1950.

Soft ice cream has grown in popularity. Today thousands of gallons are manufactured in batch and continuous freezers. In some states the composition of the soft ice cream is the same as for the regular product; in other states 3-6% fat is permitted and the serum solids are usually about 14-16%, with 15-16% sugar.

Machines have been made to break down hard ice cream. The product made by one of these machines (the Sof-Tec) has been studied at the Univ. of Illinois. The product should be from 6-8° F. when placed in the machine, if too warm, the finished product is too thin and if too cold, the motor will be overworked. The cup should be cold at the start of the operation and the final temperature of the product should be about 17-18° F. A 12% product is too rich and filling for some customers. The lower fat product has proven to be popular. Fruits, syrups and crisp breakfast foods can be added to the machine along with the frozen product.

The final over run usually is 50-55% regardless of the over run of the original product. The amount of emulsifier used in the frozen product has no relation to the over run in the final product.

For low fat products (5-6%), the serum solids should be about 15-16% and sweetening agents containing monosaccharides should not be used. For ice milk type of products, about 2 times as much emulsifier and about one-third more stabilizer should be used than for regular ice cream

W. H. Martin

**315. Sandwiches, mass production.** V. M. RABUFFO. *Ice Cream Trade J.*, **46**, 1: 36. Jan., 1950.

Automatic equipment for mass production of ice cream sandwiches has been developed by the General Ice Cream Co., Schenectady, N. Y. A wafer dispensing unit is synchronized with the flow of ice cream from the extruder attachment to the freezer and both are synchronized with the speed of a slowly moving conveyor belt.

From a sloping tray, wafers are fed contin-

uously one at a time on to the conveyor belt as it moves along. At the next station, ice cream is extruded in a continuous flow in ribbon-like form over the wafers. Next, wafers are fed from a second sloping tray to the top of the layers of ice cream. The entire string of sandwiches moves along the conveyor belt; at the end of the belt a girl "breaks off" the individual sandwiches and places them in a tray. The tray moves to the hardening room and after 1 hr. the sandwiches are bagged and boxed.

The sandwich conveyor setup weighs 290 lb. The dispensing unit weighs 130 lb. and is set on casters so it can be moved readily when not in use.

W. H. Martin

**316. Apparatus for serving ice cream.** H. W. PROTZELLER. (Assignor of one half to A. W. Nelson.) U. S. Patent 2,495,077. 3 claims. Jan. 17, 1950. *Official Gaz. U. S. Pat. Office*, **630**, 3: 823. 1950.

Ice cream is hardened in the shape of rods or cylinders and dropped into this device end to end in a vertical position. The chamber holding the ice cream is separated from a refrigerated cylinder by a small air space. A sliding valve in the bottom cuts off a segment of ice cream when depressed and releases same through an opening in the bottom when the valve is returned to its normal position.

R. Whitaker

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**317. Quality improvement programs.** C. J. BABCOCK, PMA, USDA. *Milk Plant Monthly*, **39**, 1: 44-46. Jan., 1950.

The ineffectiveness of quality improvement programs is due to buyers who will purchase low quality raw materials, lack of sincerity in setting up programs and improper approaches to producers. Effective programs must be set up on an area basis and, following improvement, the area should be used as a demonstration area to instigate future programs. Cooperation of plants and uniform grading also are essential to improved quality. Although competent fieldmen can do much to improve quality, leadership cannot be delegated but must be retained by the manufacturers of dairy products. J. A. Meiser, Jr.

**318. The shifting emphasis in quality control of milk supplies.** A. C. FAX, II. P. Hood and Sons, Boston, Mass. *Milk Plant Monthly*, **39**, 1: 26-30. Jan., 1950.

The author traces the history of quality control of the nation's milk supply during the past dec-



ades and presents a discussion of the 3 factors generally credited with promoting this progress, namely, (a) development, perfection and application of new quality tests, (b) advancements in dairy engineering and processing methods and (c) more rigid inspection of production and processing facilities. These changes have been affected by the succeeding generations of new thinkers in the dairy industry. J. A. Meiser, Jr.

**319. Housewife complaints on milk flavors.** J. A. NELSON, Montana State College, Bozeman. Milk Plant Monthly, **38**, 12: 46-47. Dec., 1949.

Flavor defects in milk are classified as those resulting from faulty production and faulty processing techniques. A third class of off-flavors are those due to circumstances outside the control of the producer or processor. The author then presents a discussion of these 3 classes of off-flavors and lists methods for preventing their occurrence in milk. J. A. Meiser, Jr.

**320. Supplementing fluid cream with frozen cream.** H. V. ATHERTON, Univ. of Vermont, Burlington. Milk Plant Monthly, **39**, 1: 22, 24-25. Jan., 1950.

In an attempt to add the maximum amount of frozen cream to fluid cream and still produce a product suitable for commercial use, the following manufacturing procedure resulted in a product nearest to that of normal fresh cream. Frozen cream combined with fresh cream on a 50-50 basis was heated to 140° F., homogenized at 100 lb. pressure, cooled and stored for 24 hr. at 40° F. The resulting product when examined for feathering, oiling-off, viscosity, blendability, plug formation and whipability was not superior to normal fresh cream but did present a usable product that may allow a more economical usage of surplus cream than is possible at present.

J. A. Meiser, Jr.

**321. Tie-in boosts cream sales.** F. FLAGG. Milk Plant Monthly, **38**, 11: 52-53. Nov., 1949.

To offset drops in cream sales during winter months, combination packages which included 1 lb. of frozen strawberries and 0.5 pt. of cream were made up. Offered at their regular retail prices, this item was featured as a week-end special and resulted in cream sales increasing 100%. Although local retail grocers complained that this sales promotion idea was detrimental to their sales, it was later proven by an accurate check that

their retail sales of berries and cream also had increased. J. A. Meiser, Jr.

**322. Container for milk and the like.** M. O. KUHN. (Assignor to The Firestone Tire and Rubber Co.) U. S. Patent 2,495,110. 2 claims. Jan. 17, 1950. Official Gaz. U. S. Pat. Office, **630**, 3: 831. 1950.

Structural details are given covering the Firestone milk can recently placed on the market.

R. Whitaker

Also see abs. no. 272, 273, 285, 286, 293, 294, 295.

## NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

**323. The food value of milk and dairy products for human consumption and means of increasing their consumption.** ETHEL A. MARTIN, National Dairy Council, Chicago. Milk Plant Monthly, **38**, 11: 42-44, 46, 48, 78. Nov., 1949.

Based on the findings of the nutrition research, the author presents a summary of currently accepted nutritional facts about milk, butter, ice cream and cheese. With this knowledge at hand, the National Dairy Council attempts to increase the consumption of dairy products by radio, research, literature, motion pictures, exhibits and leader contacts. J. A. Meiser, Jr.

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**324. Substantial savings by a planned equipment cleaning program.** K. L. FOWLER. Milk Plant Monthly, **39**, 1: 54-56. Jan., 1950.

A planned equipment cleaning program that has provided substantial savings includes: (a) dispensing of cleaning compounds in paper bags from a centralized point, (b) preparation of cleaning solutions in a centralized tank from which they may be dispersed through pipe lines to the cleaning operations, (c) maintaining hot water at 115-120° F. and dispensing at 25-40 lb. pressure, (d) phosphoric acid in preference to abrasives for milkstone and stain removal, (e) chlorine sterilization of vats by means of an air pressure spray gun and (f) use of nylon brushes and specialized cleaning aids for small-parts washing. J. A. Meiser, Jr.

Also see abs. no. 291, 305.

# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
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and the Milk Industry Foundation

### BOOK REVIEWS

**325. The Market Milk Industry.** C. L. ROADHOUSE AND J. L. HENDERSON. 2nd ed. McGraw Hill Book Co., Inc., New York, N. Y. 716 pp. \$7.00. 1950.

This widely used text seems to have been thoroughly revised to incorporate postwar changes in methods and added knowledge in the field. Bulk collection of milk from the farm which has been going on for some time in the authors' state, California, is treated in some detail. More space is given to the STIIT system of pasteurization, newer cleaning and sterilizing materials, homogenized milk and several newer products such as frozen milk and cream, plastic cream, hydrated milk, recombined milk and yoghurt.

The volume is a complete treatise on the market milk industry. Farm production conditions, especially as they affect the esthetic and sanitary quality of the milk, are well covered. Besides milk plant construction and operation, both distribution and pricing plans show the effects of recent thinking. The portion on the milk plant laboratory and its operation should prove helpful to every person concerned with laboratory control.

The volume contains 171 illustrations most of which readers will recognize as new. The index covers 25 pages.

E. F. Goss

**326. Manual for the Cheese Industry.** J. C. MARQUARDT, Marquardt Publishing Co., Geneva, N. Y. 91 pp. \$3.00.

This manual is a compact booklet which briefly outlines making procedures for such types of cheese as American, cottage and cream, as well as process cheese, process cheese food and process cheese spreads. No attempt is made to discuss the fundamentals of cheesemaking.

Very little information is given on the difficulties normally encountered in cheesemaking or methods of solving the more common types of manufacturing problems. Brief discussions on milk composition, plant sanitation, starter making and calculations are included. The author states that it was his aim to condense the manual so that facts of immediate concern with better cheese-

making are at hand. This manual will be of particular interest to those desiring a "thumb nail" sketch of the cheese industry and those wanting a quick, handy reference to the more common types of making procedures.

H. E. Calbert

**327. Industrial Microbiology.** 2nd ed. S. C. PRESCOTT AND C. G. DUNN. McGraw-Hill Book Co., Inc., New York, N. Y. xii + 923 pp. \$8.50. 1949.

Considerable revision and expansion has been incorporated in this new edition. The treatment of the yeasts (primarily those of industrial importance for carbohydrate utilization) has been enlarged and rearranged. Considerable information on newer fermentations has been added. Material on utilization of whey for production of ethyl alcohol has been incorporated. Five new chapters, including one on antibiotics, have been added. Most of the material on lactic acid fermentations, fermented milks, cheese and other food products remains unchanged from the first edition.

F. E. Nelson

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**328. The brucella ring test in mixed raw milk supplies.** H. E. BREMER, Vt. Dept. Agr., Montpelier, Vt. *Am. J. Pub. Health*, 40, 3: 290-292. Mar., 1950.

Composite samples were taken from the producers' milk cans to avoid vat contamination. Where the herd milk showed a positive or suspicious reaction with the ring test, a blood test on the individuals in the herd would reveal positive or suspicious animals. Complete comparisons could not be made since not all the herd showing such positive or suspicious ring tests were blood tested. The author concludes that the ring test is of value in helping to improve the safety of the raw milk supply and since it is much less expensive than the blood test, it can be applied more often to detect early herd infection.

D. D. Deane

- 329. The possibility of disease-free herds.** J. C. BUXTON. *J. Soc. Dairy Technol.*, 2, 4: 201-207. July, 1949.

The general methods of detecting and controlling diseases of cattle are discussed. Emphasis is placed on brucellosis, tuberculosis, Johne's disease and mastitis. E. M. Foster

- 330. Method and article for treatment of mammary glands.** F. E. MARTIN. U. S. Patent 2,498,374. 10 claims. Feb. 21, 1950. Official Gaz. U. S. Pat. Office, 631, 3: 818. 1950.

For treating mastitis, an antibiotic-containing material which is rigid at normal temperature is dispersed into the teat canal by being forced out of a tube-like device which is inserted in the teat opening. R. Whitaker

## BUTTER

O. F. HUNZIKER, SECTION EDITOR

- 331. Aluminum foil wraps for print butter.** A. H. WHITE. *Can. Dairy Ice Cream J.*, 29, 2: 46-52. Feb., 1950.

The new foil wrapper which has been developed in Canada and United States consists of a thin 0.0045" aluminum foil laminated to a light-weight parchment of 15 lb./ream. In some cases, the parchment has been of 27 lb. weight. The foil usually is treated with a protective coating to prevent corrosion from brine. The aluminum foil wrapper combines the good characteristics of vegetable parchment with the added protective qualities of the impermeability of aluminum foil to water, vapor, odors and light. Results indicate that the foil wrapper proved to be much superior to parchment alone in preventing deterioration of the surfaces of prints, absorption of foreign odors and flavors, action of sunlight, loss of weight and color changes at the surface. Some disadvantages of the foil wrap are (a) extra cost; (b) the sharp edge of the foil may cause cuts when butter is wrapped by hand; (c) the foil may become wrinkled and lose some of its attractiveness; (d) foil does not give as much physical protection to butter as parchment plus a waxed carton.

H. Pyenson

- 332. Sediment testing of cream and butter.** J. D. INGLE, Swift and Co., Chicago. *Natl. Butter Cheese J.*, 41, 2: 28-29. Feb., 1950.

The ideal method of making the sediment test is one that will work on all types of cream, will not impair the quality of the cream or alter its churning properties due to added material and will be rapid enough so that it does not delay factory operations.

The condition of the protein in sour cream makes sediment testing of this product difficult. Field tests showed that use of a 10% solution of sodium citrate or a 3% solution of phosphoric acid was most effective in conditioning the cream so that pt. samples could be filtered without undue delay.

Compared with cream, the sediment testing of butter is relatively simple. Seven-cm. filter paper is recommended for laboratory use when a careful microscopic examination of butter sediment is to be made. H. E. Calbert

- 333. Butter package.** C. G. BENNETT. (Assignor to Paterson Pacific Parchment Co.). U. S. Patent 2,497,203. 2 claims. Feb. 14, 1950. Official Gaz. U. S. Pat. Office, 631, 2: 418. 1950

A sheet of moisture-proof material and a sheet of water-permeable material, laminated together with a layer of mold-retarding substance, such as Na or Ca propionate with salt, between the sheets, is used as a wrapper for butter. The moisture-proof side of the wrapper is on the outside. R. Whitaker

## CHEESE

A. C. DAHLBERG, SECTION EDITOR

- 334. Present-day methods of Stilton cheese manufacture.** T. J. BRINDLEY. *J. Soc. Dairy Technol.*, 3, 1: 13-15. Oct., 1949.

Two methods used to make Stilton cheese are described briefly. In the method used in some of the larger factories the curd is cut in 0.5-in. cubes and allowed to remain undisturbed in the vat for about 4 hr. After the whey is drained off, the curd is ladled onto perforated drainers in sinks, allowed to settle and cut into 4-in. blocks. The next morning the curd is broken apart, milled, salted and filled into perforated hoops. The cheeses are turned at intervals for about 5 or 6 d. to facilitate even drainage, after which they are moved to a cool, well ventilated room to dry the surface. The cheeses are pierced with needles at weekly intervals for 3 wk. beginning at about 18 wk. after manufacture. Inoculation of the curd with mold spores is left to chance.

The method in general use is essentially similar to that described above except in handling the curd. In this method the curd is not cut but is ladled soon after its formation into coarse linen cloths supported on laths or wires. As drainage progresses the cloths are tied tighter and tighter until the curd is sufficiently firm to transfer onto drainers, after which it is handled as described above. E. M. Foster

**335. History of the Stilton cheese industry.** J. G. W. STAFFORD. *J. Soc. Dairy Technol.*, 3, 1: 11-13. Oct., 1949.

The origin of Stilton cheese is described.

E. M. Foster

Also see abs. no. 326, 327, 343, 352.

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**336. Vacreator as a milk evaporating unit.** R. W. BROWN AND J. J. JANZEN. *Can. Dairy Ice Cream J.*, 29, 1: 56-62, 76. Jan., 1950.

The vacreator has been used as an evaporating unit at the Dairy Science Department at the University of Manitoba during the summers of 1946, 1947 and 1948. 261,131 lb. of skim milk and buttermilk were evaporated at approximately a 3.12:1 ratio. The product was circulated through the vacreator at 160-165° F. By concentrating the product to 24.0% milk solids instead of to 28.0%, the loss of solids in the processing was reduced by an average of more than 50%. The net return per 100 lb. of skim milk and buttermilk amounted to over \$1.00 or approximately 5 times the usual value placed on these products. The vacreator-produced skim milk and skim milk and buttermilk mixtures were found to be preferable to skim milk powder of unspecified characteristics when used in ice cream mixes.

H. Pyenson

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**337. The incidence of coliform and milk souring organisms in Welsh farm and creamery water supplies.** PATRICIA M. FRANKLIN AND GWYNETH GEORGE. *J. Soc. Dairy Technol.*, 2, 4: 220-222. July, 1949.

Examination of a large number of dairy water supplies during the past 20 yr. showed that 82% of the farm dairy water samples, 55% of the country creamery samples and 28% of the town dairy samples contained more than 2 coliform bacteria/100 ml. Organisms capable of producing an acid curd in litmus milk incubated at 30° C. for 48 hr. were found in practically all samples that contained coliform bacteria and in some samples that lacked coliform organisms.

E. M. Foster

**338. A note on the use of the colony count at 22° C. in assessing the suitability of a water supply for dairy purposes.** G. E. JONES. *J. Soc. Dairy Technol.*, 2, 4: 222-223. July, 1949.

The absence of coliform bacteria does not guar-

antee the suitability of a water supply for dairy use. The author suggests inclusion of a plate count in the bacteriological examination of such water supplies to detect undesirable organisms other than coliform bacteria. He suggests tentatively the use of standard nutrient agar as the medium with incubation for 3 d. at 22° C.

E. M. Foster

**339. The types of bacteria commonly found in farm and creamery water supplies and their action on milk and milk products.** S. B. THOMAS. *J. Soc. Dairy Technol.*, 2, 4: 224-232. July, 1949.

The author presents a general discussion of the types of bacteria likely to be found in water supplies and the effects they may cause based on his study of 242 farm water supplies; it is supplemented generously by reference to pertinent published articles. The danger of using untreated water for washing dairy utensils, plant equipment, butter, etc. is stressed particularly from the standpoint of the introduction of spoilage organisms.

E. M. Foster

**340. Bacteriological colony counting device having a light-conducting member for transverse illumination.** R. M. WOOD. U. S. Patent 2,495,912. 6 claims. Jan. 31, 1950. *Official Gaz. U. S. Pat. Office*, 630, 5: 1230. 1950.

To facilitate counting the colonies on a petri plate, this counter is so designed that light from a lamp is reflected from the polished inside wall of a globular-shaped bowl beneath the plate and illuminates the colonies transversely from all sides. A mask under the dish prevents direct rays of light from shining through the dish from beneath.

R. Whitaker

**341. The "5-minute" resazurin test for determining the quality of raw milk.** J. C. BOYD AND H. C. HANSEN, Univ. of Idaho, Moscow. *J. Milk and Food Technol.*, 13, 1: 40-43. Jan.-Feb., 1950.

The resazurin test using 5, 10 and 15 min. periods was compared to the methylene blue test. The results showed the 5-min. reading to be the most accurate in grading poor milk. Resazurin "B" grade milk in 5 min. showed 71.9% of the samples had direct microscopic counts ranging from 21,000,000 to 60,000,000/ml.

The authors suggest that the resazurin test should be supplemented by other tests, preferably the direct microscopic count. The "5-min." resazurin test is valuable when the milk will reduce methylene blue in 3 hr. or less.

H. H. Weiser

**342. Preliminary observations on the application of an alcohol screening test, used in con-**

junction with the ten-minute resazurin test, for detecting unsatisfactory milk on arrival at creameries. ANN E. HUGHES AND DOROTHY ELLISON, National Milk Testing Service, Brynawel, Aberystwyth. J. Soc. Dairy Technol., 2, 3: 149-151. Apr., 1949.

Alcohol strengths of 76, 72 and 68% were compared with the 10-min. resazurin test for detecting poor quality milk in 378 samples tested during the summer of 1948. Relatively good agreement was obtained between the alcohol tests and the samples showing good quality by the resazurin test. However, 27% of the samples showing poor quality by the resazurin test were not detected by the 76% alcohol, and an even greater percentage was missed by the lower alcohol concentrations. The authors feel that even though a high proportion of poor quality milks is missed by the 76% alcohol test it is still more reliable than visual and olfactory examination by a plant worker.

E. M. Foster

343. The bacteriophage of cheese cultures. C. E. PARMELEE, F. E. NELSON, G. E. TURNER AND P. H. CARR. Iowa Agr. Expt. Sta., Ames. Natl. Butter Cheese J., 41, 1: 28-30. Jan., 1950.

Acid production virtually is stopped in single strain cultures when they are attacked by bacteriophage. In mixed cultures an attack by bacteriophage is characterized by slow acid production. Bacteriophage particles cannot be seen with the ordinary microscope. By use of the electron microscope they appear to be sperm-shaped, 220  $m\mu$  long, with a head 70  $m\mu$  in diameter. The tail is approximately 150  $m\mu$  long and 30  $m\mu$  wide.

A simple method of determining relative numbers of bacteriophage particles in an infected material is to add a measured amount of susceptible culture plus a measured amount of properly diluted material to tubes of sterile skim milk. Incubate tubes 12-14 hr. at 32° C. Under these conditions the control containing only organisms will be coagulated. The presence of bacteriophage is indicated when there is no coagulation in the tubes of lower dilution. The amount of bacteriophage particles present in the suspected material can be judged by the highest dilution that evidences bacteriophage activity.

Another method for determining relative numbers of bacteriophage particles depends on the production of clear areas or plaques on agar plates. There are several illustrations of this method.

H. E. Calbert

344. The occurrence of *Proteus* spp. in raw and pasteurized milk. A. J. ZARETT, R. N. DOETSCH AND P. ARNE HANSEN. Univ. of Md.,

College Park. J. Milk and Food Technol., 13, 1: 31-34. Jan.-Feb., 1950.

The frequency of the *Proteus* group of organisms in the intestinal tract has been reported by several investigators. Obviously milk carelessly handled likely will contain human and animal excreta. It has been suggested that *Proteus* serve as a test organism to indicate pollution in milk instead of the *Escherichia-Aerobacter* group. The use of *Proteus* would have the added advantage in that no species differentiation would be necessary, since each species would have some sanitary significance. The use of urea-ricinoleate agar culture medium to detect *Proteus* colonies was satisfactory and necessitated no further biochemical tests.

H. H. Weiser

345. Pyridoxal phosphate and pyridoxamine phosphate as growth factors for lactic acid bacteria. W. S. McNUTT AND E. E. SNELL, Univ. of Wis., Madison. J. Biol. Chem., 182, 2: 557-567. Feb., 1950.

In a medium shown to be complete for the growth of most lactic acid bacteria, 1 species each of *Lactobacillus helveticus*, *L. acidophilus* and *L. delbrueckii* grew only when such natural materials as malt sprouts or autolyzed yeast were included in the medium. Observations made during the fractionation and concentration of malt sprout extract led to tests of the growth-promoting effect of phosphate esters of light-sensitive vitamins. For maximum growth of *L. helveticus* and *L. acidophilus*, 1  $m\gamma$  of pyridoxamine phosphate/ml. medium is equivalent to 200  $\gamma$  malt sprouts provided any one of the following substances was included: non-specific reducing agents, vitamin B<sub>12</sub> and thymidine or other desoxyriboside. A 7-fold concentration of pyridoxal phosphate could substitute for pyridoxamine phosphate; both vitamin B<sub>6</sub> phosphates could be replaced by D-alanine.

Except for a specific requirement for thymidine in these experiments, the strain of *L. delbrueckii* responded similarly to phosphorylated vitamin B<sub>6</sub> and D-alanine.

Pyridoxamine, pyridoxal and L-alanine were inactive at the concentrations tested. Coenzyme I and II, flavin-adenine dinucleotide, cocarboxylase and various nucleotides exhibited very low activity or were inactive.

H. J. Peppler

346. Competitive antagonism of ribonucleic and desoxyribonucleic acids in the nutrition of *Lactobacillus bifidus*. H. R. SKEGGS, J. SPIZZEN AND L. E. WRIGHT, Sharpe and Dohme, Inc., Glenolden, Pa. J. Am. Chem. Soc., 72, 2: 811-813. Feb., 1950.

*Lactobacillus bifidus* (ATCC4963), recently reclassified as *L. acidophilus*, is capable of utilizing

either thymidine or vitamin B<sub>12</sub> for growth in an otherwise complete medium. In the absence of either of these substances, this organism responds to increasing amounts of intact deoxyribonucleic acid (DNA) over a range of 0.5 to 5  $\gamma$ /ml. This lactobacillus is more sensitive to DNA as an essential nutrient than to vitamin B<sub>12</sub>. Ribonucleic acid (RNA) competitively inhibits the utilization of DNA by *L. acidophilus*. In view of the essential nature and widespread occurrence of DNA in all living cells, the specific blocking of DNA utilization by RNA could be of great importance in many studies of cellular metabolism.

H. J. Peppler

Also see abs. no. 327, 328, 365, 384.

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**347. Isolation of DDT from fats.** B. DAVIDOW, Food and Drug Administration, Federal Security Agency, Washington 25, D. C. J. Assoc. Off. Agr. Chemists, 33, 1: 130-132. 1950.

The conventional Schechter-Haller colorimetric method for determination of DDT was not applicable in the presence of more than traces of fat, and the isolation of DDT from large quantities of fat has remained a serious analytical problem.

The author outlines in detail a more convenient and faster method for the separation of fat from DDT. Celite (commercial diatomaceous earth) impregnated with sulfuric acid-fuming sulfuric acid and slurried with carbon tetrachloride holds fats within a chromatographic column while DDT passed through with the carbon tetrachloride. Recoveries of microgram quantities of DDT from 5 g. of butteroil ranged from 90 to 100%.

F. J. Babel

**348. Effect of thymus nucleate on the thermal coagulation of albumin solutions.** J. P. GREENSTEIN AND M. L. HOYER, Nat. Cancer Inst., Bethesda, Md. J. Biol. Chem., 182, 2: 457-466. Feb., 1950.

The coagulation of bovine and horse serum albumin and chicken egg albumin in aqueous solutions (pH 6.4-6.7) was prevented by relatively small amounts of sodium thymus nucleate provided both albumin and nucleate solutions are practically salt-free. One mg. of nucleate nearly completely protects about 150 mg. albumin against coagulation at 98° C. for 10 min. The degree of coagulation of the heated protein was nearly linear with decreasing amounts of nucleate. Yeast nucleate has no apparent effect on albumin, even at relatively high concentrations. Unlike other agents known to increase the ther-

mal stability of albumin, thymus nucleate does not prevent denaturation on heating, but only coagulation. When heated solutions of albumin and protecting nucleate were treated with deoxyribonuclease and magnesium ions, coagulation of the heated protein gradually ensued as the protecting nucleate was digested.

H. J. Peppler

**349. Manufacture of lactalbumin.** E. C. SCOTT AND G. W. McDONALD (Assignors to Swift and Co.). U. S. Patent 2,497,420. 2 claims. Feb. 14, 1950. Official Gaz. U. S. Pat. Office, 631, 2: 473. 1950.

The acidity of whey is adjusted to between 0.07 and 0.12% calculated as lactic acid. It then is heated to a temperature at which the albumin normally coagulates, but does not under these conditions. With minimum agitation there is added to the quiescent heated whey additional acid to bring the whey to 0.15-0.3% calculated as lactic acid. This causes the lactalbumin to precipitate suddenly in large floating lumps, from which the whey readily is drained.

R. Whitaker

**350. The sedimentation constant, diffusion constant and molecular weight of lactoglobulin.** R. CECIL AND A. G. OGSTON, Univ. of Oxford. Biochem. J., 44, 1: 33-35. 1949.

Sedimentation and diffusion constants were made on 3 preparations of lactoglobulin and from these values a molecular weight of 35,400 was calculated. This figure is in good agreement with other reported values. There is some discussion regarding differences observed.

A. O. Call

**351. Physics in the dairy industry.** R. HARPER, Nat. Inst. for Research in Dairying, Univ. Reading, England. J. Soc. Dairy Technol., 3, 1: 39-45. Oct., 1949.

The physical characteristics of milk, cream, butter and cheese are discussed. Normal values for viscosity, density, size of fat globules, freezing point, refractive index and surface tension are given for milk and, in some cases, for cream.

E. M. Foster

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**352. Apparatus for agitating curds.** P. P. CAUMARTIN. U. S. Patent 2,496,001. 3 claims. Jan. 31, 1950. Official Gaz. U. S. Pat. Office, 630, 5: 1253. 1950.

This agitator has 2 flanged rollers which roll along the top of the sides of the cheese vat and support an arm which extends down into the vat.

Attached to the lower end of the arm is a paddle wheel-shaped curd agitator, driven by a chain and sprocket from a power source above the vat.

R. Whitaker

**353. Instrumentation for high temperature short time pasteurization.** W. S. YOUNG, The Foxboro Co., Foxboro, Mass. *Am. Milk Rev.*, 11, 10: 26-29, 50, 51. Oct., 1949.

Instruments for high-temperature, short-time pasteurization include heater temperature controller, flow diversion valve operator, cold milk recorder and brine temperature controller. An electronic timer for determining holding time by timing a heat impulse is described. The design and mechanics of operation of the flow diversion valve are explained.

D. J. Hankinson

**354. Routine maintenance of short time high temperature control systems.** R. E. OLSON, Taylor Instrument Co, Rochester, N. Y. *Am. Milk Rev.*, 11, 10: 33, 57. Oct., 1949.

Periodic checking of high-temperature, short-time equipment should include (a) cleaning air valve in pressure controller, (b) blowing out all air filters daily, (c) cleaning air valve in temperature controller, (d) checking accuracy of safety thermal limit recorder, (e) examining rubber disc rings in flow diversion valve for wear and leakage and (f) examining all air pipe connections for leaks.

The cause and correction of the following troubles are indicated: (a) Lag in reaching fixed milk temperature, (b) wandering of final milk temperature, (c) continuous "hunting" of final milk temperature, (d) failure of flow diversion valve to operate when milk is at correct temperature, (e) failure of flow diversion valve to remain in forward flow position and (f) failure of milk pump to start.

D. J. Hankinson

**355. Refrigeration in manufacture of ice cream.** H. M. TYRRELL. *Can. Dairy Ice Cream J.*, 29, 1: 27-29, 74. Jan., 1950.

In order to have sufficient compressor capacity, one should know what refrigeration is required at the temperature that one wishes to operate. For example, a 10-ton machine will do 10 tons only at 19.6 lb. back pressure. About 10% of freezer troubles are due to refrigeration. New freezers should not be piped up to existing suction and liquid lines to the compressor as the lines usually are too small to take care of the additional load. The installation of an ammonia still is an effective way of cleaning the system of oil and "dead" liquid. In the hardening room, ice cream hardens in about half the time when properly stacked and when proper blast fans are used rather than still

air. The proper oil should be selected for the ammonia compressor for the temperature range at which the compressor is to operate. Brine and brine systems should be kept at pH 9 and at the proper specific gravity to give the necessary freezing point. Sodium chromate inhibits corrosion in brines. In all plants operating on a low back pressure, it is necessary to install an automatic purger.

H. Pyenson

**356. Dairy refrigeration.** F. N. BEAMS. *J. Soc. Dairy Technol.*, 2, 2: 115-121. Jan., 1949.

The basic principles of refrigeration are described briefly along with some of the common types of refrigerating systems. The application of refrigerating equipment to cooling and storage of fluid milk and ice cream are discussed.

E. M. Foster

**357. Standards have to grow.** F. A. FAUST, The Bristol Co., Waterbury Conn. *Am. Milk Rev.*, 11, 10: 42, 43. Oct., 1949.

The 3A standards have been established which have eliminated threaded construction on instrument fittings in contact with milk. Other subjects under investigation include homogenizer flange, cold milk recorder specifications, storage tank sockets, "blister bulb" construction for storage tank controllers, specifications for high-temperature, short-time pasteurizers and thermal timing methods for determining holding time for high-temperature, short-time equipment.

D. J. Hankinson

**358. Electronic instruments for dairy processors.** J. MEYER, Minneapolis-Honeywell Regulator Co., Philadelphia, Pa. *Am. Milk Rev.*, 11, 10: 34, 49. Oct., 1949.

The applications of the pyrometer (thermocouple plus potentiometer) are discussed. Multi-point recorders offer certain advantages for control work. Such electronic equipment offers an obtainable accuracy of 0.125° F. and is difficult to break and easy to fix. Instruments not now available may be offered to the industry in the future.

D. J. Hankinson

**359. Possibilities in the sterilization of milk by means of radiations.** H. BURTON. *J. Soc. Dairy Technol.*, 2, 2: 75-80. Jan., 1949.

The possible applications of radio frequency waves, ultraviolet rays, X-rays, gamma rays, cathode rays, alpha particles, neutrons and sonic and ultrasonic waves in the treatment of milk are discussed with references to pertinent literature. The author believes that of the methods listed only ultraviolet radiation seems to be approaching the stage where commercial utilization is possible.

E. M. Foster

**360. Method of sterilizing and preserving.** A. BRASCH (Assignor to Electronized Chemicals Corp.). U. S. Patent Reissue 23,195. 17 claims. Feb. 7, 1950. Official Gaz. U. S. Pat. Office, 631, 1: 79. 1950.

Reissue of U. S. Patent 2,456,909 covering sterilization of milk and other foods by bombardment with high speed electrons of a velocity of 1 million volts for not over 0.000001 sec

R. Whitaker

**361. Temperature and pressure instruments in the dairy industry.** A. LEBOUTILLIER, Taylor Instrument Co., Rochester, N. Y. Am. Milk Rev., 11, 10: 8-10, 55. Oct., 1949.

The Bourdon tube as a temperature and pressure measuring device is described. Various temperature control systems are briefly discussed, including on-off control, proportional response, automatic reset and the self-acting regulator. The application of these control systems to the short-time, high-temperature pasteurizer is explained.

D. J. Hankinson

**362. Bourdon pressure spring.** J. W. BEECHER, The Bristol Co., Waterbury, Conn. Am. Milk Rev., 11, 10: 12, 56. Oct., 1949.

The design and manufacture of Bourdon pressure spring for use in pressure and temperature measuring instruments is discussed.

D. J. Hankinson

Also see abs. no. 336.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**363. Waste prevention in the dairy industry.** E. F. ELDRIDGE. Milk Dealer, 39, 5: 49, 50, 88-94. Feb., 1950.

This is a report of the Task Committee on Dairy Waste Disposal of the Dairy Industry Committee. The average stream contains from 7-10 parts of oxygen per million parts of water. Wastes from dairy plants deplete this oxygen until streams cannot maintain normal aquatic life and thus, unpleasant odors, black sludge deposits and gray fungus growth result. The oxygen from 1,600 gal. or normal, unpolluted stream water is required to decompose the organic material in 1 pt. of milk. The average milk receiving station with good housekeeping methods can keep its milk waste to limits of 4 lb. of biological oxygen demand /10,000 lb. of milk, or a milk loss of 0.35%. The following sources of waste in dairy plants and methods of preventing them are discussed: (a) Leakage, (b) overflow, (c) spillage,

(d) freezing on, (e) willful waste, (f) residual waste and (g) carry over. Data is presented showing the approximate average composition of milk and milk products, the BOD and population equivalent per hundredweight. The most important processes in the dairy industry and the pounds BOD loss which can be expected from each process with reasonably modern equipment and careful operation also is shown. A waste prevention program is discussed.

C. J. Babcock

**364. Cutting clerical costs.** Anonymous. Milk Dealer, 39, 4: 43, 70-72. Jan., 1950.

Pet Dairy Products Co. of Greensboro, N. C., has cut clerical costs on accounts receivable by one-half through use of photographic equipment. The advantages of the system are: (a) The customer has a completely detailed, neat invoice. (b) The milk dealer has a full record of each transaction on an easily-indexed, ready reference film roll. (c) Time-and-a-half of clerical effort has been completely freed for other activity. (d) Duplication of effort is materially reduced. (e) Bills are out sooner, and collections are better. (f) Follow-up of inactive accounts is surer and faster.

The microfilm rolls lend themselves to simple but complete indexing; reference to current or old records is fast. The reader, in addition to presenting an image in sharp, clear enlargement also serves as a fast, inexpensive printer, should a copy of the statement, invoice or other filmed document be required.

C. J. Babcock

Also see abs. no. 385, 388.

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**365. Preliminary observations upon factors influencing cellulose digestion by rumen microorganisms.** W. BURROUGHS, N. A. FRANK, P. GERLAUGH, AND R. M. BETHKE, Dept. of Animal Industries, Ohio Agr. Expt. Station, Reynoldsburg. J. Nutrition, 40, 1: 9-24. Jan., 1950.

Cellulose digestion by rumen microorganisms was carried out in 500-ml. glass containers at 40° C. Filter paper was the source of cellulose. The starting inoculum was a mixed culture of organisms taken directly from rumens. CO<sub>2</sub> was bubbled through the liquid. Digestion was allowed to proceed for 36 hr. at which time 1/2 of the fermentation medium was removed, with the remaining amount serving as an inoculum for a succeeding 36-hr. digestion period. The portion removed was analyzed to determine cellulose digestion. Those additions to the fermentation media which favored cellulose digestion were a complex salt solution, ash of alfalfa extract, auto-



claved rumen liquid and autoclaved water extract of manure. R. K. Waugh

**366. Yellow gas from corn silage.** W. H. PETERSON, R. W. THOMA AND R. F. ANDERSON, Univ. of Wis. *Hoard's Dairyman*, **94**, 23: 870-871. Dec. 10, 1949.

Reports of yellow gas, fatal to chickens and flies, around the bottom of a silo were investigated. A sample of such gas was obtained from the silo room at the bottom of the chute of one of the University of Wisconsin silos. The sample was found to contain both nitrites and nitrates. Nitrogen dioxide at the rate of 151 parts per million were found. The yellow gas is believed to come from the reduction of nitrates in the corn. The production of yellow gas stops after a few days. No danger from the use of this silage is expected. A. R. Porter

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

**367. Proving sires and dams.** Anonymous. *Hoard's Dairyman*, **94**, 23: 872-873. Dec. 10, 1949.

The report of a committee of the Purebred Dairy Cattle Association to set up uniform rules for proving sires and dams is given. Seven points in the recommended procedure of proving sires and dams are listed. A preliminary proof on sires is recommended with the first 5 daughter-dam comparisons and a proof should be reported with 10 or more comparisons. The recommended proving of a dam includes weighted values of records of the cow, her daughters and her sons' daughters. A. R. Porter

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**368. Milk flow controls for milking machines.** A. G. PERKINS. U. S. Patent 2,496,307. 23 claims. Feb. 7, 1950. Official Gaz. U. S. Pat. Office, **631**, 1: 92. 1950.

A spring-loaded valve is described which controls the milk flow from a vacuum operated milking machine, cutting off the vacuum when the milking operation is complete. R. Whitaker

**369. Supporting means for milking apparatus.** S. DALY (Assignor to International Harvester Co.). U. S. Patent 2,497,299. 5 claims. Feb. 14, 1950. Official Gaz. U. S. Pat. Office, **631**, 2: 442. 1950.

A stand equipped with rack and pinion allows adjustment of a milk collecting vessel below the

udder. The teat cups are attached with relatively short hose connections. R. Whitaker

**370. Milking system and apparatus therefor.** G. R. DUNCAN. U. S. Patent 2,498,401. 14 claims. Feb. 21, 1950. Official Gaz. U. S. Pat. Office, **631**, 3: 824. 1950.

Milk cans standing in a refrigerated cabinet are filled directly from a milking machine through a device located in the cover of the cans which permits discharge of the milk with no loss of vacuum. R. Whitaker

Also see abs. no. 398.

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**371. Ice cream—paper containers in relation to shrinkage.** J. A. MEISER, Mich. State College, East Lansing. *Sou. Dairy Prod. J.*, **47**, 3: 30-31. Mar., 1950.

Results of the experiment reported indicate that containers in themselves will not necessarily prevent shrinkage, but certain treatments of the containers may retard the volume loss to some extent. Coating paper containers with paraffin or glassine definitely lessened the degree of shrinkage of ice cream. F. W. Bennett

**372. Formulas for making sherbets with whey on a commercial scale.** F. E. PORTER AND D. H. WILLIAMS, U. S. D. A., Washington, D. C. *Ice Cream Trade J.*, **46**, 2: 52. Feb., 1950; *Ice Cream Rev.*, **33**, 7: 53. Feb., 1950.

See abs. no. 218.

**373. The manufacture of culturized ice cream.** W. H. E. REID, Univ. of Missouri, Columbia, AND C. B. AGEE, R. M. HANCKEL AND R. H. THOMAS. *Sou. Dairy Products J.*, **47**, 3: 34, 122-124. Mar., 1950.

The effects of the addition of a dehydrated culture of lactic acid bacteria in de-fatted milk in ice cream were studied. The acidity of the dehydrated culture was standardized to a pH slightly above that of non-fat dry milk solids, prior to dehydration by the addition of a standardizing agent "Minsol."

Dehydrated culture used as a source of serum solids imparted a distinctive culture flavor to ice cream. Mixes containing 3 and 5% of dehydrated culture were superior in smoothness and mellowness of body and closeness of texture. Dehydrated culture showed unusual stabilizing properties and when added at the rate of 5% of the total weight of the mix, tended to cause the resulting ice cream to be too stable. F. W. Bennett

**374. Fruit strainer.** J. B. ORRELL (Assignor to Abbotts Dairies, Inc.). U. S. Patent 2,496,636. 6 claims. Feb. 7, 1950. Official Gaz. U. S. Pat. Office, 631, 1: 178. 1950.

This device separates the juice from the pulp of fruit for flavoring ice cream, etc. A screw conveyor, operating in a perforated cylinder within a closed inclined cylinder of larger diameter, propels the pulp along to a discharge hole, while the juice escapes through the perforations and is collected between the 2 cylinders and is removed through a bottom drain. R. Whitaker

**375. Cocoa products for ice cream.** L. FREUNDLICH. Hooton Chocolate Co., Newark, N. J. Ice Cream Trade J., 46, 2: 62, 63, 72. Feb., 1950.

Choice of flavoring material may depend on the price relationship of milk fat and cocoa butter, also on whether the manufacturer has an homogenizer. If no homogenizer is available, it is best to use cocoa, because it is easier to disperse it into a syrup than it is to disperse chocolate liquor. Less stabilizer is recommended for chocolate ice cream than for vanilla, and the higher the fat content of the chocolate flavoring the less stabilizer will be needed.

Cocoa should be free from gritty matter. All of it should pass through a 100 mesh sieve and at least 97% through a 200 mesh sieve. The finer the powder, the better will be its flavor. The numerous varieties of cocoa beans each has its characteristic color and flavoring. Chocolate and cocoa manufacturers blend different cocoas to get the desired color and flavor characteristics for ice cream.

Color intensity in chocolate ice cream may be due to variation in density, manner of dipping shrinkage or change in acidity due to bacterial activity. W. H. Martin

**376. Ice cream disher.** S. BLOOMFIELD. U. S. Patent 2,498,331. 2 claims. Feb. 21, 1950. Official Gaz. U. S. Pat. Office, 631, 3: 807. 1950.

This is a modification of the well known semi-circular ice cream disher with a blade which rotates within the bowl to discharge the ice cream. The novel feature is the ratchet arrangement which rotates the shaft attached to the discharging blade. R. Whitaker

**377. Melvern's "prepacked" low overrun, bulk pint.** Ice Cream Trade J., 46, 2: 42, 106. Feb., 1950.

Melvrens Dairies, Inc., of Washington, D. C., are marketing a factory-filled, pail-type package of ice cream which contains 14% fat and 43% overrun retailing at 35¢/pt. and costing dealers

26¢. All pre-packed bulk pints are sold in solid flavors in specially designed cartons. In order to freeze the ice cream with 43% overrun, it is necessary to reduce the freezer capacity one-third. W. H. Martin

**378. Producing sandwiches in volume with low labor costs.** Anonymous. Ice Cream Trade J., 46, 2: 66. Feb., 1950.

A potential process developed by LeRoy Foods, Inc., Brooklyn, N. Y., makes possible production of 400 doz. ice cream sandwiches an hour at a cost of about 1¢/doz. Sandwich wafers 2.75 in. square placed in individual special cartons holding 3.5 fluid oz. are used. Units of 24 are filled with ice cream directly from the freezer and go on to the hardening room. W. H. Martin

**379. Recent developments in making ice cream novelties.** E. J. OTKEN, Good Humor Corp., Brooklyn, N. Y. Ice Cream Trade J., 46, 2: 50. Feb., 1950.

Production of chocolate-coated ice cream stick confection has grown to 3-3.5 billion annually, valued at \$300,000,000. Details of current production practices are given. W. H. Martin

**380. The flaming nut sundae.** Anonymous. Ice Cream Trade J., 46, 2: 46. Feb., 1950.

The flaming nut sundae was introduced by the Liggett-Rexall Drug Store Chain. A pint of vanilla ice cream is cut in half lengthwise and one-half placed in a banana split dish. Hot fudge (1½ oz.) then is streamed lengthwise over the ice cream and 1 tablespoon of pecan bits is sprinkled over this. Half a marshmallow is placed in the center with a cube of sugar freshly moistened with 3 drops of lemon extract on it. The extract, containing about 80% alcohol, burns brightly when lighted. The sundae sells for 29¢. W. H. Martin

**381. 1949 gallonage.** Anonymous. Ice Cream Trade J., 46, 2: 44, 98. Feb., 1950.

According to figures released by the U. S. D. A., the estimated 1949 production of ice cream was 553,650,000 gal., 3% below the 1948 volume. New York State's production was 61,440,000 gal., an increase of 5 million gal. over that of 1948. Pennsylvania produced 65,380,000 gal., a slight increase over 1948. W. H. Martin

**382. The new national drug store survey.** Ice Cream Trade J., 46, 2: 74-82. Feb., 1950.

See abs. no. 302.

Also see abs. no. 355, 356, 391.

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**383. "Trouble shooting" in quality control work.** W. A. CORDES, National Dairy Products Co., Inc. *Milk Dealer*, 39, 4: 41, 102. Jan., 1950.

A discussion is given of quality defects in dairy products which are a result of the following factors: (a) poor quality of raw materials, (b) lack of proper processing equipment to do the job, (c) failure of equipment to do the job for which it is designed, (d) lack of proper facilities and supplies, (e) failure of personnel to use equipment properly to do the best possible job with the facilities available, and (f) failure of management and supervision to supply personnel with directions and formulas and to instruct, supervise and check personnel. The necessity of training and experience is emphasized. C. J. Babcock

**384. Screening and rejection tests in relation to the keeping quality of milk.** ELEANOR JONES-EVANS. *J. Soc. Dairy Technol.*, 2, 4: 232-236. July, 1949.

A 72% alcohol test and the 10-min. resazurin test were compared on 1,341 samples of milk taken at 9 creameries in mid- and south Wales during April-Sept., 1948. Accuracy of the tests in detecting milk of poor quality was checked by comparing with keeping quality of the samples as determined by holding at 20° C. and performing clot-on-boiling tests at intervals.

The alcohol screening test was judged unreliable because it passed as satisfactory a high proportion of samples with keeping quality times less than 12 hr. Samples judged unsatisfactory by the 10-min. resazurin test (disc numbers 0-3.5) usually had unsatisfactory keeping times (less than 12 hr.). However, samples with higher disc number (4-6) showed a wide range of keeping quality times. It was concluded that disc readings of 4-6 on the 10-min. resazurin test gave little indication of the actual keeping quality of the milk. E. M. Foster

**385. Can the Northeast forget manufactured milk?** E. O. MATHER, Dellwood Dairies, Yonkers, N. Y. *Am. Milk Rev.*, 11, 11: 2-4, 55. Nov., 1949.

The formula method of determining the price of Class I (fluid) milk in the Boston market is unrelated to the price of milk for manufactured dairy products. The New York Class I-A price is tied to the Boston Class I price. Until 1948, the price of milk for fluid purposes was approximately \$1.25/cwt. over the midwestern condenser price. In the 10-mo. period Sept., 1949, through June, 1949, the New York Class I-A price

was \$2.49/cwt. or almost double the historical price. The problems resulting from this price relationship are (a) increase in milk production causing lowered blend price, (b) increased pressure from outside producers to get into the pool, causing a lowered blend price, (c) very low prices for surplus milk which is in competition with midwestern cream and butter and (d) consumption of evaporated milk encouraged at the expense of fluid milk. Furthermore, with formula pricing of Class I (fluid) milk according to economic conditions and with government support for manufactured dairy products, it is possible that northeastern producers might experience a very low return if economic conditions become serious. D. J. Hankinson

**386. The consumer looks at a bottle of milk.** MRS. MILDRED MESKIL, N. Y. State Dept. of Commerce, Albany, N. Y. *Milk Dealer*, 39, 4: 46, 78-82. Jan., 1950.

A questionnaire distributed to consumers in 3 major up-state New York urban areas reveals that ¾ of all the people interviewed had milk delivered at home and 24.6 of the remaining 25% purchased milk in some form or other, usually from the local store. Most consumers demanded regular delivery and 93% agreed they got it but 22% were not satisfied with the delivery time. There was an overwhelming preference for square glass containers. Fifty-six per cent did not want the 2-qt. bottle and many of those who would use it would expect a price reduction. Sixty-two per cent indicated that they found it desirable to purchase other commodities from the milkman. In the low income group, 60% bought milk because it was good for them, while in the middle income group 78.8% bought for this reason. In the first group, 63.3% used it in cooking; the latter group bettered this figure by 10%. Fifty per cent of all respondents bought because it was good for the children and 45% simply because it tastes good. Three-fourths of all who answered were familiar with homogenized milk; half of these purchased it and of that total 86% prefer it. The only objection to homogenized milk was no "top milk" for coffee or cereal use. Some 98.5% of those replying use fresh milk; 43.6% use evaporated milk; 37.9% use cream; 27.9% use chocolate milk and 1.7% use powdered milk. C. J. Babcock

**387. What do consumers think of bottled fresh concentrated milk?** G. M. TROUT AND G. G. QUANKENBUSH, Michigan State College, East Lansing. *Am. Milk Rev.*, 11, 11: 42-45, 55. Nov., 1949.

See abs. no. 147.

**388. Milk tokens.** P. G. KEMP. *Can. Dairy Ice Cream J.*, **29**, 1: 33, 71. Jan., 1950.

The cost of milk tokens is only about 25% of the cost of tickets. There is a savings of the cashier's time and the route salesman's time. Customers have accepted tokens about 99.5%. Milk tokens easily drop out of a damp milk bottle. Large stocks of tokens are not needed as they are kept in circulation. On a 10,000 qt. of milk/month basis, only 1,500 milk tokens would be required for each product. Counterfeiting has not yet been a problem. H. Pyenson

Also see abs. no. 325, 341, 342, 344, 357, 359, 360.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**389. Some recent researches on milk secretion.** H. D. KAY. *J. Soc. Dairy Technol.*, **3**, 1: 17-21. Oct., 1949.

A discussion of the role of hormones in milk secretion is presented. E. M. Foster

## NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

**390. Food value of milk and dairy products.** E. A. MARTIN. *Can. Dairy Ice Cream J.*, **29**, 2: 66-76, 88. Feb., 1950.

This paper was delivered by the author at the 12th International Dairy Congress, Stockholm, Sweden, 1949. It covers rather completely the food value of milk, butter, cheese, whey and ice cream and means of increasing their consumption through nutritional research projects, printed literature, motion pictures, advertising, convention exhibits, publicity and leader contacts. H. Pyenson

**391. Ice cream in the field of nutrition.** H. D. BRANION. *Can. Dairy Ice Cream J.*, **29**, 2: 39-41, 90. Feb., 1950.

Ice cream has been shown to be a food and not a confection. Assays and analyses on ice cream from a nutritional angle are few. Ice cream stands out for mineral calcium, riboflavin and vitamin A content. Canadian dietary often lacks calcium and riboflavin and by increasing the consumption of ice cream more would be obtained. H. Pyenson

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**392. The response of the ovary of the anestrus goat to pregnant mares' serum gonadotrophin.**

S. J. FOLLEY, A. L. GREENBAUM AND A. ROY, *Nat. Inst. for Research in Dairying, Univ. of Reading, England. J. Endocrinol.*, **6**, 2: 121-131. Oct., 1949.

Goats during the anestrus season were brought into estrus by the injection of pregnant mares' serum (p.m.s.) and subsequently mated. Of 35 goats injected with a single subcutaneous dosage of 1200 i.u. of p.m.s. and brought into heat, only 22.8% produced young following copulation. This low conception rate could be accounted for in part by a lowered fertility of the male during the anestrus season. No differences in response were observed in comparing subcutaneous to intravenous hormone dosage. The dosage used induced super ovulation but no concomitant superfetation was noted. In some cases, estrus did not follow p.m.s. injection although ovulation was known to have occurred. V. Hurst

**393. Influence of variation in environmental temperature and thyroid status on growth and feed consumption of the male mouse.** M. MAQSOOD AND E. P. REINEKE, *Mich. State College, East Lansing. Am. J. Physiol.*, **160**, 2: 253-258. Feb., 1950.

Growing male mice were studied under environmental temperatures of 24 and 30° C. with a relative humidity held between 45-55%. Control mice were fed Purina laboratory chow and experimental mice were fed the chow plus varying amounts of thiouracil or thyroprotein or combinations of thiouracil and thyroprotein. Feed and water consumption were measured.

Control mice at 30° C. gained less weight than control mice at 24° C. Thiouracil feeding depressed growth at both temperatures. A dosage of thyroprotein (0.025%) which stimulated growth at 24° C. caused growth depression at 30° C. Growth stimulation by feeding thyroprotein could be induced at 30° C. but the stimulating dosage had to be reduced to 0.005% of the ration.

Thiouracil feeding lowered both food and water consumption at high and low temperatures. Thyroprotein feeding increased food intake in proportion to dosage even when increased dosages meant decreased body gains as compared to controls. V. Hurst

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**394. Equipment cleaning programs.** K. R. FOWLER, *National Dairy Products Co., Inc., New York, N. Y. Ice Cream Trade J.*, **46**, 2: 60, 114. Feb., 1950.

Cleaning compounds should be weighed or

measured into bags and dispensed to the people doing the cleaning. One of the most economical ways of making up a cleaning solution is to use a centralized solution tank from which the heated solution may be piped to points of use and then dispensed through 0.25-in. hoses, with shut-off valves onto equipment to be cleaned. This method may save from \$600-\$4,000/yr., depending on the size of the plant.

A hot water generator or instantaneous system, in conjunction with temperature controls, water pressure regulators, 0.5-in. hose and shut-off valves will cut down fuel consumption as much as 50% over the common method of mixing steam and cold water. A water pressure of 25-40 lb. at the hose and a temperature of 115-120° F. will do an effective job of cleaning.

Phosphoric acid has been found more satisfactory for removal of milk stone than scouring powders. Sterilization is accomplished by using liquid chlorine, 10-15% available chlorine made up 220 ppm for spraying, 100 ppm for brushing and 50 ppm for flowing through pipes, pumps and vats. A spray gun with air pressure is the most effective means for sterilizing vats, tanks, coolers and other open surfaces.

Nylon-filled brushes have proved much more effective and less expensive in the long run to the conventional type of cleaning brushes.

W. H. Martin

**395. The cleaning and sterilization of milk plants.** A. ROWLANDS, Nat. Inst. for Research in Dairying, Univ. Reading, England. *J. Soc. Dairy Technol.*, 2, 3: 152-156. Apr., 1949.

The principles of cleaning and sterilizing dairy equipment are discussed along with the applications of various methods. The uses of steam, hot water, chlorine, alkalies and quaternary ammonium compounds are considered briefly.

E. M. Foster

**396. The importance of efficient washing of utensils for steam and hypochlorite sterilization.** A. H. WALTERS, C. M. COUSINS AND S. B. EDMUNDS. *J. Soc. Dairy Technol.*, 2, 3: 136-139. Apr., 1949.

A comparison of the bacteriological quality of milk produced on 2 farms during the summer of 1947 showed that high quality milk could be produced with either steam or hypochlorite sterilization of utensils provided the equipment was cleaned well before sterilization. One of the farms practiced machine milking, the other hand

milking. Both used steam sterilization during the 1st half of the test period and hypochlorite during the latter half. Poor quality milk was produced only during the time when utensils were improperly cleaned before sterilization.

E. M. Foster

**397. Chemistry of cleaning farm milking equipment.** D. LEVOWITZ, N. J. Dairy Laboratories, New Brunswick, N. J. *Milk Dealer*, 39, 4: 104-107. Jan., 1950.

The chemistry of cleaning utensils used in handling milk is discussed to show that the practice of "rinsing milking utensils with cold water, directly after use" has needlessly complicated dairy farmers' lives and caused the production of oceans of poor milk; milk residues can be perfectly removed from utensil surfaces by dispersing them with appropriate wetting agent solutions, then flushing with plain water.

C. J. Babcock

**398. The sanitizing of milking machines.** A. C. DAHLBERG, F. V. KOSIKOWSKY, H. W. SEELEY AND A. A. LEUENTHAL. Cornell Univ., Ithaca, N. Y. *J. Milk and Food Technol.*, 13, 1: 5-18, 24. Jan.-Feb., 1950.

Good sanitizing results were obtained with neutral nonionic detergent and quaternary ammonium compounds when they were used with accepted sanitary procedures. When used together, they were not good solvents of dirt on milker rubber tubes. Quaternary ammonium compounds used alone or with nonionic detergents were not satisfactory in sterilizing unclean rubbers, but the combination gave good results when the rubbers were brush-washed in a hot solution. C1 solution was not as effective as lye when used as a solvent cleaner and sterilizer. The sterilization action of the alkaline and the quaternary compounds was increased by the alkaline solution. If a pH from 9.5-10.5 was not maintained in the washing solution, the quaternaries failed to destroy *Pseudomonas* organisms.

A satisfactory sanitizer for cleaning and sterilizing was suggested. It contained tetrasodium pyrophosphate, trisodium phosphate, sodium carbonate, a nonionic surface active detergent and a quaternary ammonium compound. A solution of this mixture had a pH of 10.5 and was very effective as a sanitizer for dairy utensils.

H. H. Weiser

Also see abs. no. 347.

# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**399. Experimental studies on the incubation period of infectious abortion in cattle.** A. THOMSEN, State Veterinary Serum Laboratory, Copenhagen, Denmark. *Brit. Vet. J.*, **106**, 2: 41-54. Feb., 1950.

A study was made on the incubation period of bovine brucellosis and antibody formation by utilizing 24 mature heifers from brucellosis-free herds. Five heifers were infected at service by a bull in which infectious material (ground placenta in saline) was introduced in the prepuce just before breeding. The remaining animals were infected orally and by eye at various periods of gestation. The length of the incubation period was proportioned to the degree of development of the fetus at the time of infection. The average incubation period was 225 d. in heifers infected at breeding but only 53 d. in a heifer infected at 7 mo. gestation. The younger the fetus at the time of infection the longer is the period of incubation. The range of the incubation period varied from 44-251 d., depending upon the stage of gestation. A series of 23 graphs and 1 table are presented to show the data more effectively.

B. B. Morgan

**400. In vitro diffusion of penicillin from penicillin ointments for bovine mastitis.** V. P. SEEBERG and J. P. STREET, Cutter Laboratories, Berkeley, Cal. *Vet. Med.*, **45**, 4: 167-169. Apr., 1950.

*In vitro* diffusion studies were made on 14 different penicillin ointments used for bovine mastitis treatment. Optimum diffusion of penicillin occurred from those with bases composed of mixtures of white and liquid petrolatum. Concentration of the white petrolatum should be between 25-50%. Addition of beeswax greatly retarded the diffusion.

B. B. Morgan

**401. Mastitis from a veterinary practitioner's viewpoint.** P. GAMBREL, Winnebago, Illinois. *Vet. Med.*, **45**, 3: 122-124. Mar., 1950.

This is a review of the mastitis problem by a veterinary practitioner. The control of mastitis should be a joint responsibility of both dairyman and veterinarian. Some points are given in favor of the suspended-type milking machine over the claw-type milker for aid in the control of mastitis. It is suggested that the predisposing factor is trauma or injury to the upper part of the teat lining. This trauma is produced by improper use of a milking machine. All of the observations were based on 7 yr. experience in veterinary practice.

B. B. Morgan

**402. The recognition of tick-borne fever as a disease of cattle.** J. R. HUDSON, Ministry of Agriculture Veterinary Laboratory, Weybridge, England. *Brit. Vet. J.*, **106**, 1: 3-17. Jan., 1950.

A mild, febrile disease of English cattle caused by a *Rickettsia* is described. The organism appears similar to *R. bovis* or *R. ovina*. Transmission studies were not made; however, the tick, *Ixodes ricinus*, has been incriminated. In cows inoculated with the organism, the incubation period ranged from 4-11 d. Although a mild disease which may upset milk production temporarily, it is not considered of much economic importance. Complete protocols are given.

B. B. Morgan

**403. An outbreak of bovine leptospirosis in Pennsylvania.** R. B. LITTLE, J. D. BECK and J. V. McCANON, Rockefeller Inst. for Med. Research, Princeton, N. J. *Vet. Med.*, **45**, 3: 104-110. Mar., 1950.

A report is given on 7 outbreaks of bovine leptospirosis involving 33 cattle on different farms in Pennsylvania. Diagnoses were made by serological tests of the blood from infected cattle and the isolation of the organism (*Leptospira*) from guinea pigs injected with blood or milk from infected cows. The disease is characterized by

hemoglobinuria, anemia, icterus, fever abortion, loss of weight, decrease of milk production and moderate constipation. Morbidity varied from 10-50%, while the mortality rate was about 3%. Complete herd histories also were given. Recommendations for the control of this disease included (a) quarantine the herd and isolate each cow during the acute stage of the disease, (b) transfusion of 500 ml. of whole blood in sodium citrate from animals which have had the disease, (c) administration of penicillin and (d) daily administration of methenamine and sodium biphosphate. There is no evidence that the leptospira involved in these cattle is pathogenic to man; however, any outbreak in cattle should be regarded as dangerous until the organism is classified.

B. B. Morgan

**404. Infectious keratitis of cattle—a preliminary report.** H. FARLEY, I. O. KLEWER, C. C. PEARSON and L. E. FOOTE, Okla. Agr. Expt. Sta., Stillwater. *Am. J. Vet. Research*, 11, 38: 17-21. Jan., 1950

*Hemophilus bovis* has been isolated from numerous eyes afflicted with infectious keratitis and has been proposed as the cause of the disease. To test this hypothesis, 34 susceptible cattle were treated with pure cultures of *H. bovis* secured from infected eyes. None of the 34 developed keratitis. Of 29 calves treated with virulent secretions from keratitis cases, 8 developed the disease. *H. bovis* did not produce keratitis in rabbits or guinea pigs either. It is likely that *H. bovis* is not the primary etiologic agent in infectious keratitis, but only a secondary invader.

E. W. Swanson

**405. The transmission of anaplasmosis.** G. DIKMANS, U.S.D.A., Beltsville, Md. *Am. J. Vet. Research*, 11, 38: 5-16. Jan., 1950.

A review of the various reported methods of transmitting anaplasmosis is presented. Transmission by ticks of widely differing species has been reported. However, very few of them support transovarian transmission, so the natural mode of transmission by ticks yet is obscure. The transfer of blood or infected material by needles, dehorning instruments, pitchforks, scalpels, etc. has been definitely responsible for numerous outbreaks. Transmission by flies, mosquitoes or other insects has been successful only when an infected animal or carrier has been in or near the susceptible herd so the insects can go directly from the infected to the susceptible animal.

E. W. Swanson

**406. Q fever studies in southern California. XI. Recovery of *Coxiella burnetii* from milk of sheep.** W. L. JELLISON, Rocky Mtn. Lab., Hamilton, Mont.; H. H. WELSH, Calif. State Dept. of Pub. Health; B. E. ELSON, Calif. State Dept. of Agr.; and R. J. HUEBNER, Natl. Institute of Health. *Pub. Health Rept.*, 65, 12: 395-399. Mar. 24, 1950.

A study of 300 cases of Q fever in southern California revealed 2 case histories with contacts suggesting sheep as the source of infection. The complement fixation test was run on 128 sheep in the flock to which 1 of these 2 patients had been exposed and only 33 were completely negative. The remainder gave low titer reactions, with none showing a positive reaction at a dilution greater than 1:16. Q fever was produced in guinea pigs by injecting lacteal secretions obtained from these sheep. A strain of *Coxiella burnetii* was established in guinea pigs and identified by accepted criteria.

D. D. Deane

**407. Problems in cattle practice.** W. J. GRIBONS, Auburn, Ala. *Vet. Med.*, 45, 4: 147-150. Apr., 1950.

A brief review is given of some of the common ailments of cattle encountered over a 2-yr. period at the large animal surgical and ambulatory clinics at the Alabama Polytechnic Institute. Some of the disease conditions encountered were arsenic poisoning, anaplasmosis, acetoneemia, bloat, malnutrition, mastitis, mycotic stomatitis, parasitisms, photosensitization, milk fever, sterility, screw worms and hyperkeratosis (X disease). Diseases discussed in detail were mycotic stomatitis, soremouth, milk fever, acetoneemia and mastitis.

B. B. Morgan

**408. Omentectomy of cattle for studying insecticide residue in the body.** R. D. RADELEFF, Bureau of Animal Industry, U.S.D.A., Kerrville, Texas. *Vet. Med.*, 45, 3: 125-128. Mar., 1950.

An operation is described for removing portions of the omentum for chemical analyses. It has been shown that some of the newer insecticides are absorbed and stored in the fat of the omentum. This material may be collected and analyzed in the laboratory for the presence of chlorinated hydrocarbons. The choice of anesthesia is important. Chloral hydrate or chloroform cannot be used since their presence may obscure analyses. A 2% procaine hydrochloride is recommended. Of over 500 operations, only 2 terminated fatally. Repeated operations apparently do not interfere with the general health or weight gains of the animal.

B. B. Morgan

## BUTTER

O. F. HUNZIKER, SECTION EDITOR

409. **Dairy engineering in the butter industry.** A. M. PEARSON. *Can. Dairy Ice Cream J.*, 29, 3: 66-70. Mar., 1950.

The success of the operation of continuous butter making machines when processing neutralized cream is due to the "de-sludge" separator bowl. Formerly, 80% fat concentration was obtained only with sweet cream. A new stainless steel, No. 20, which is supposed to be more corrosion resistant than 18-8 has been introduced. Aluminum production has increased and cost has decreased so that more uses may be found for the metal in the dairy industry. Greater emphasis has been placed of late on the sanitary condition of equipment used in the manufacture of butter. Wooden butter equipment is being replaced by metal equipment. In the past few years the small, compact steam generator or packaged boiler has been used as the sole source of heat in the plant. In refrigeration the trend seems to be toward decentralization of refrigeration plants and towards multicylinder compressors operating at much increased RPM. Freon as a refrigerant seems to be gaining in popularity.

H. Pyenson

## CHEESE

A. C. DAHLBERG, SECTION EDITOR

410. **Cracked rinds in cheddar cheese.** E. G. HOOD AND C. A. GIBSON. *Can. Dairy Ice Cream J.*, 29, 3: 29-30. Mar., 1950.

Cracked rinds in cheddar cheese may be caused by: (a) the development of excessively dry body; (b) high acidity; (c) low acidity due to inactive starter; (d) greasy curd; (e) temperature of curd at time of hooping under 70° F.; (f) use of previous day's curd; (g) hot water treatment in the hoop; (h) clogged press cloths and (i) failure of bandage and circle to meet.

H. Pyenson

411. **Cheddar cheese storage plants.** O. R. IRVINE. *Can. Dairy Ice Cream J.*, 29, 3: 90-94. Mar., 1950.

A major cost in assembling and storing cheese is that for labor. To reduce labor costs newer plants in Illinois and Wisconsin have gone to a 1-floor operation, except where processed cheese was being manufactured. Other labor costs can be saved by using fork trucks, storing cheese on pallets, high ceilings in storage rooms free from posts and blower coils for cooling. Advantage

should be taken of automatic control equipment for refrigeration machinery. In the installation of insulating materials special attention is now being given to vapor barriers. H. Pyenson

412. **Några iakttagelser vid paraffinering samt runmärkning av ost. (Some studies on the paraffining and Rune-branding of cheese.)** G. LARSSON, Svenska Mejeriernas Riksförening Meddelande, 4. Dec., 1947.

An automatic paraffining machine manufactured by the Wedholm factory, Model GE, was used in the experiments. The machine had 3 forks and each fork was designed to lower 1 cheese into the paraffin until it was well coated and then lift it to the table. Herrgård, svecia and gouda cheese were used. In 1 hr. 200 cheese could be coated. The temperature of the paraffin was held at 150° C. for these experiments.

Some experiments in which the paraffining machine was not used were made to determine how long a time the cheese could be immersed in the paraffin without being damaged. The cheese could be in the hot paraffin for up to 10 sec. with no noticeable damage to it. A table was prepared to show the results from the experiments.

For the labeling of cheese with the "Rune" or Swedish national brand, an ordinary electric flat-iron was used. At the experimental dairy plant at Örnköldsvik a test was made to determine the cost of labor. Three persons could label 100 cheese in 185 min., using 2 labels on each cheese. By using only 1 label and by arranging the work to save time and labor, the working time could be reduced about 35%. G. H. Wilster

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

413. **Variations in the bactericidal and bacteriostatic properties of milk.** A. D. McEWEN AND MARJORY B. WHITE, Moredun Institute, Edinburgh. *Vet. Record*, 62, 3: 27-30. Jan. 21, 1950.

This experiment was designed to determine if fluctuations in the bactericidal properties of milk occur which may account for degrees of resistance of the udder to infection. *Str. pyogenes* was the test organism used. Samples were taken from the R.H. and L.H. quarters of 1 cow for approximately a 2-mo. period during which time the R. H. quarter was injected with sterile saline, *Str. uberis* and *Str. dysgalactiae*. Mild inflammation caused by these injections did not alter the bactericidal and bacteriostatic titers of the milk. Studies on a different cow showed



the titers of the milk did not change with season or ration. Several cows showed a decrease in titers during the drying off period, but results did not indicate a difference between intermittent milking or complete cessation as a method of drying.

R. P. Niedermeier

**414. Preparing and maintaining good cultures.** N. C. ANGEVINE, Meyer-Blanke Co. Milk Plant Monthly, 39, 3: 30-32. Mar., 1950.

Milk used in preparing cultures must be of excellent quality and contain 10-13% solids. Temperatures of at least 185° F. and holding periods of 30 min. to 1 hr. are necessary in obtaining proper pasteurization. Amount of inoculant will range from 0.25-2%, depending on the activity of the mother culture. Following incubation periods of 14-16 hr. at 70-72° F. good cultures will possess a smooth, firm texture, free of whey or gas, and an acidity of 0.80-0.85%. Cultures should also have a pleasant clean flavor that is mild but not pronounced.

J. A. Meiser, Jr.

**415. Penicillin and other starter inhibitors.** H. A. RUEHE, Univ. of Illinois, Urbana. Milk Plant Monthly, 39, 3: 36, 38-39, 42. Mar., 1950.

Faulty cultures and delayed coagulation of cottage cheese may be caused by: (a) bacteriophage, (b) germicidal agents in cans and utensils and (c) antibiotics. Recently veterinarians have been injecting antibiotics such as penicillin into udders in the treatment of mastitis. To determine the effect of this drug on the quality of milk, as measured by the methylene blue reduction test, milks of high and low quality were inoculated with 4, 20, 80 and 160 units of penicillin/100 ml. of milk and methylene blue tests run on the samples. Presence of penicillin retarded decolorization of methylene blue but not sufficiently to change the classification of the milk. A suggested method for determining the presence of penicillin in milk involves pasteurization of a 10-ml. sample at 175° F. for 5 min., followed by cooling to 72° F. and inoculation with 1 ml. of starter. If no penicillin is present, a satisfactory coagulum should form in 10 hr. or less.

J. A. Meiser, Jr.

**416.  $\alpha$ -Acetolactic acid, an intermediate in acetylmethylcarbinol formation.** (Abs.) E. J. JUNE, Western Reserve Univ., Cleveland, O. Federation Proc., 9, 1: 396. Mar., 1950.

Substantial evidence was obtained supporting the theory that acetolactic acid is the critical intermediate in the bacteriological conversion of pyruvic acid to acetylmethylcarbinol. A crude

extract of *Aerobacter aerogenes* was resolved into 2 components, 1 capable of acting on pyruvic acid to form acetolactic acid and CO<sub>2</sub>, the other decarboxylating acetolactic acid to acetylmethylcarbinol but without effect on pyruvic acid. Enzymatically produced acetolactic acid was shown to be identical with synthetic acetolactic acid. These findings were found to be consistent with the activity of preparations from other microorganisms.

S. Patton

**417. The nutrition of variants of *Lactobacillus bifidus*.** R. M. TOMARELLI, R. F. NORRIS AND P. GYORGY, Univ. of Pennsylvania, Philadelphia, and J. B. HASSINEN AND F. W. BERNHART, Wyeth, Inc., Mason, Mich. J. Biol. Chem., 181, 2: 879-887. Dec., 1949.

*L. bifidus*, a predominant organism in the intestinal tract of infants fed human milk, grows better in culture media having milk added. Human milk was better than cow's milk. The growth factor in milk is associated with the unsaturated fatty acid fraction. Skimming did not decrease the activity. When both milks were digested with pancreatin, they showed an increase in growth activity and became equal in effect. A concentrate of the active constituents was obtained but not identified. At high levels, the digested cow's milk was inhibitory, but not so with the digested human milk. Both were found to contain steam-distillable inhibitory substances, but the digested human milk in addition had protective factors which neutralized the inhibitor. Growth factors for some of the strains of *L. bifidus* isolated are discussed.

A. O. Call

**418. Inhibition of growth of *Lactobacillus casei* by methionine and its relation to folic acid assimilation.** G. TOENNIES, H. M. WINEGARD and D. L. GALLANT, Lankenau Hospital Research Institute and The Institute for Cancer Research, Philadelphia. Arch. Biochem., 25, 2: 246-256. Feb., 1950.

The role of methionine in the nutrition of *Lactobacillus casei* (ATCC 7469) has been studied with a view to clarification of contradictory findings reported by others. In the synthetic medium of Evans, as little as 10  $\gamma$  of L-methionine/ml. medium effectively prolongs the lag phase of *L. casei* through its interference with the assimilation of folacin. During the early phases of growth the organisms were shown to hoard folacin; after accumulation has ceased, methionine was observed to accelerate growth by serving as a protein building block, sparing cystine. The inhibitory effect of methionine is considered to be a case of nutrient antagonism and is

unique in that it involves a vitamin and an amino acid, is not reversibly competitive, and the inhibitory concentration of amino acid is of a lower order of magnitude than that which is nutritionally effective. H. J. Peppler

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**419. A use of ascorbic acid in frozen homogenized milk.** R. B. ANDERSON, C. W. BETZOLD and W. J. CARR, Sixth Army Area Food Laboratory, Seattle, Wash. *Milk Plant Monthly*, 39, 3: 74-77. Mar., 1950.

To prevent the development of oxidized flavor concentrations of ascorbic acid ranging from 1.5 to 12.0 g. were added to 100 lb. of milk. Following pasteurization for 15.8 sec. at 164° F. and homogenization under 1,700 lb. pressure, the milk was cooled to 38° F. and stored in ice cream-style, deep freeze units at 0° F. for periods ranging from 30-90 d. Results indicated that 6 g. of added crystalline ascorbic acid/100 lb. of milk retarded the development of oxidized flavors and enabled the milk to retain appreciable amounts of ascorbic acid for 90-d. storage periods. Milk fortified to the 6-g. level that was properly handled could be shipped long distances and still be a good source of vitamin C.

J. A. Meiser, Jr.

**420. Note sur le dosage des chlorures dans les laits. (A note on the determination of chlorides in milks.)** M. DURON and A. FOURNIER. *Lait*, 30, 291-292: 33-34. Jan.-Feb., 1950.

The procedure of Massot and Lestra for the determination of chlorides in milk is endorsed mainly because of sharpness in titration endpoint. Some minor modifications of the method, which appear to be advantageous, are presented.

S. Patton

**421. Nouvelles recherches sur l'enzyme de Schardinger. (New studies of Schardinger's enzyme, xanthine or aldehyde oxidase.)** L. M. BURUANA. *Lait*, 30, 291-292: 2-24. Jan.-Feb., 1950.

A rather thorough review of known facts concerning the enzyme is presented at the outset. For the purposes of the study a method of separating the enzyme from milk was developed. The method appears to be convenient and to yield an enzyme extract of high strength and purity; essentially, it consists of extracting cream, which has been exhaustively washed by centrifuging and decantation of wash water, with a small quantity of 1%  $\text{NaHCO}_3$  solution.

Methods of preserving and properties of the enzyme extract prepared according to this procedure are given. Activity of the enzyme preparation is positively correlated with its P content. Enzyme activity was measured as time necessary to decolorize a standard methylene blue solution containing a known quantity of benzaldehyde.

The mechanism of the enzyme action in milk is elaborated by oxidation-reduction potential measurements using platinum and calomel electrodes. Donation of hydrogen by an aldehyde and acceptance of hydrogen by methylene blue or nitrates is demonstrated. Mathematical considerations are given relating the reduction phenomenon to an equation,  $V = e^{ft/t'}$ , where  $V$  equals the calomel electrode potential and  $ft$  is a function of time and variable temperature coefficients.

S. Patton

**422. The quantities of amino acids in the non-protein fraction of breast and cow's milk.** R. J. BLOCK and D. BOLLING, N. Y. Medical College, New York. *Arch. Biochem.*, 25, 2: 350-353. Feb., 1950.

The approximate essential amino acid distribution in the nonprotein fractions of cow's milk (dry-skimmed and evaporated-skimmed milk) and human milk (3 mo. or longer post partum) was determined chemically and compared with the previously-reported amino acid patterns of the total proteins. Although breast milk contains about 4 times as much nonprotein nitrogen as does cow's milk, the essential amino acid patterns in the nonprotein fractions, as well as the total proteins, of the 2 milks show no significant differences. Calculations in terms of mg.% amino acids in milk or colostrum are presented to facilitate their use by nutritionists.

H. J. Peppler

**423. Microbiological determination of isoleucine in proteins and foods.** M. J. HORN, D. B. JONES and A. E. BLUM, U.S.D.A., Washington. *J. Biol. Chem.*, 180, 2: 695-701. Sept., 1949.

A microbiological method for the determination of isoleucine is described. Results in good agreement with reported values were obtained using either *L. mesenteroides* or *S. faecalis* as the test organisms. Among the proteins and foods reported are values for casein, lactalbumin and dry skim milk.

A. O. Call

**424. Standardizing the Babcock test for milk.** E. O. HERREID. *Can. Dairy Ice Cream J.*, 29, 3: 78-80. Mar., 1950.

In order to standardize the Babcock procedure and make the test more accurate, the committee

appointed in the American Dairy Science Association has revised the test. By increasing to 18 ml. the volume of the sample of milk and eliminating the meniscus by the use of glymol, the Babcock test has been made in closer agreement with the ether extraction method. The results submitted by 5 collaborators on a total of 135 samples of unpreserved milk prove that the modified Babcock method is accurate.

H. Pyenson

**425. Effects of anions, cations and amino acids on bovine alkaline phosphatases.** (Abs.) C. A. ZITTLE and E. S. DELLA MONICA, Eastern Regional Research Lab., Phila., Pa. Federation Proc., 9, 1: 251. Mar., 1950.

Studies of the activity and inhibition of alkaline phosphatases from cow's milk and from intestinal mucosa of the calf were made. Measurement of enzyme activity was based on liberation of phenol from phenylphosphate substrate. The results of the studies suggest that milk and intestinal phosphatases differ.

S. Patton

**426. Liberation of amino acids and active peptides from raw, heated and vitamin-free casein by tryptic digestion.** (Abs.) R. A. SULLIVAN, W. E. DOWNEY, E. K. STANTON, E. VAN WAGONER and M. J. HANSELL, Natl. Dairy Research Labs., Oakdale, N. Y. Federation Proc., 9, 1: 236. Mar., 1950.

Tryptic digestion revealed differences in the amount of amino acids liberated from a freshly prepared casein paste (46%), vitamin free casein (35%) and commercial casein (26%). Differences also were shown in non-dialyzable nitrogen and total weight of available amino acids in the respective hydrolysates.

S. Patton

**427. Studies on lactalbumin heated with carbohydrate.** (Abs.) I. J. MADER, L. J. SCHROEDER and A. H. SMITH, Wayne Univ., Detroit, Mich. Federation Proc., 9, 1: 365. Mar., 1950.

*In vitro* studies using pancreatin and *in vivo* studies with dogs indicated that heating lactalbumin with carbohydrate (part of the study concerning lactose) lowers the nutritive value of the protein. *In vitro* studies revealed that lysine was partially destroyed and that enzyme-resistant materials appeared to have been formed. Lowered digestibility was shown by increased fecal nitrogen in the *in vivo* studies.

S. Patton

**428. Liberation of amino acids and peptides from raw and heated bovine plasma albumin by pepsin and trypsin.** W. H. RIESEN and C. A.

ELVEHJEM, Univ. of Wisconsin, Madison. Arch. Biochem., 25, 2: 335-346. Feb., 1950.

Amino acids and peptides released from raw and heated (to render it soluble during heating) crystalline bovine plasma albumin by crystalline pepsin and trypsin were assayed microbiologically with *Leuconostoc mesenteroides* P-60 and *L. citrovorum* 8081 and compared with the total release obtained by acid or alkaline hydrolysis. The peptic release of microbiologically-utilizable peptides was unaffected by previous heating of the albumin; however, heating increased the release of active peptides by trypsin. Maximum digestion was observed in the action of the pepsin-trypsin sequence on raw albumin or the trypsin-pepsin sequence on heated albumin. The extent of the release of peptides containing amino acids utilized by the assay bacteria varied considerably. Peptides containing utilizable tyrosine, valine and methionine were released most readily by pepsin, while arginine, tryptophane and methionine were rendered most available to the bacteria by trypsin. In this respect the results agree generally with those reported in studies with casein. Only negligible quantities of free amino acids were found in the peptic and tryptic digests of raw or heated albumin.

H. J. Peppler

Also see abs. no. 442.

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**429. Emulsionsproblemer ved mælk og fløde.** (Milk and cream emulsion problems.) A. B. WITTEG. Nordisk Mejeri-Tidsskrift, 15, 7: 94-96. Aug. 15, 1949.

It is best to keep the pH of the milk product not lower than 6.6 for the production of the best emulsion by homogenization. Milk and cream should not have been subjected to storage for any length of time at temperatures of 10-50° C. before homogenization as this would cause damage to the natural emulsifier in the product. Homogenized products cannot be cooked for as long a period as unhomogenized products.

The color of milk changes from bluish-white to white during homogenization; this undoubtedly is due to the adsorption of casein during the increase of fat globule surface. The viscosity increases and the flavor becomes "fuller," richer and sweeter.

The illustrations show that the increased membrane surface area in milk as a result of the homogenization process causes an increased effectiveness in the product's emulsifying agents, a phenomenon not dissimilar to the one observed

when wetting agents are added to a fat-in-water emulsion. The article is concluded by the statement that the emulsion problems in connection with milk and cream do not cease at the dairy plant, but continue for a long time after the product has been marketed, and not least, after it has been consumed.

G. H. Wilster

**430. Milk filtration.** H. INGLESANT. *Food*, 19, 22: 25. Jan., 1950.

Experiments indicated that when modified starch was added to milk in quantities ranging from 0.1–0.5% and the milk recirculated in a plate and frame press until a filter coating developed, a useful filtration procedure was obtained. Bacteria numbers were significantly reduced; an "homogenizing effect" was noted, although fat content was not affected and extraneous matter was removed.

K. G. Weckel

**431. Practical piping problems.** R. C. SORONEN. *Heating, Piping, & Air Cond.*, 22, 3: 104–106. Mar., 1950.

Installation of piping for processing requires planning not only in the routing of mains, but also for pipeline anchors, guides, supports and hangers. Several types of anchors, guides and supports are illustrated by drawings which show means of hanging, approximate dimensions and type of material used. A schedule for spacing of piping supports and hangers is presented.

H. L. Mitten, Jr.

**432. Reviving sick compressors.** H. C. WELCH. *Operating Eng.*, 3, 3: 36–37. Mar., 1950.

A brief step-by-step procedure for overhauling a vertical, single-acting, ammonia compressor is presented. The points covered are safety precautions, pumping-out of compressor, removal of cylinder heads, crankshaft bearings, cylinders and rings, pistons, valves, wrist pins and bushings, packing and assembly.

H. L. Mitten, Jr.

**433. License requirements for stationary and marine engineers.** S. M. ELONKA, Operating Engineer, Albany, N. Y. *Operating Eng.*, 3, 2: 19–49. Feb., 1950.

The article is a tabulation of operating engineers' steam license requirements for states in the U. S., provinces in Canada, big cities of U. S. and Canada and the Merchant Marine. Headings of the requirements table are: States and cities, examination required, education and experience, class license, citizenship, local residence, non-local licenses recognized, fee and renewal and remarks.

H. L. Mitten, Jr.

Also see abs. no. 409, 411.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**434. Various forms of reduced delivery systems.** G. E. FOOTE. *Can. Dairy Ice Cream J.*, 29, 3: 35–39, 102. Mar., 1950.

In Canada and the U. S. there are 6 major delivery systems being used not counting 7-d.-a-week delivery. They are as follows in order of their usage percentage-wise: (a) every-other-day delivery (E.O.D.). Over 90% of all the home milk is delivered on this system in the U. S.; (b) 3-d.-a-week delivery; (c) 6-d.-a-week delivery (plant operation 7 d.); (d) 6-d.-a-week delivery (plant operation 6 d.); (e) 4-d.-a-week delivery; (f) 5-d.-a-week delivery. In the U. S. less than 3% of the milk delivered to the homes is via the 7-d.-a-week system. In Canada it still is being used by 90% of milk delivery markets. The advantages and disadvantages of the various systems are given in detail.

H. Pyenson

**435. Increasing plant efficiency through application of engineering practices.** L. C. THOMSEN, Univ. of Wisconsin, Madison. *Ice Cream Rev.*, 33, 8: 48, 63. Mar., 1950.

The cost of producing a gallon of vanilla ice cream with 98% overrun, containing 10% fat, 10% serum solids, 16% sugar, 0.25% egg yolk solids and 0.2% stabilizer was estimated to be \$1.146 in 1949. Of this amount, the ingredient cost amounted to \$0.5164, labor, \$0.3146, overhead, \$0.2016 and operations, \$0.1134.

The ingredient cost is based upon the approximate minimum compositional standards and maximum overrun consistent with the production of quality ice cream. Since this is no time for the industry to sacrifice quality, the possibility of reducing costs by reducing the fat content of the mix or by increasing overrun is out of the question. Any reduction in the price of mix ingredients is apt to be offset by increased labor costs.

Delivery costs have been reported to vary from a low of \$0.2757/gal. for stops using 6000–7000 gal./yr. to over \$0.90/gal. for the 100–200 gal./yr. stop. If delivery costs were to exceed \$0.33/gal., a corresponding increase in selling price would appear necessary.

In 1947, the average annual wage paid to production workers in the ice cream industry was \$2,347, as compared to \$1,108 paid in 1939. The average wage cost for production workers per gallon of ice cream has increased from \$0.057 in 1939 to \$0.106 in 1947, despite the fact that the

output per worker during this same period increased from 19,578 gal. in 1939 to 22,138 gal. in 1947. Purchase of new equipment is justified whenever the labor which it saves will pay for the equipment within 3 yr.

Overhead costs includes depreciation, taxes, insurance, donations, travel, advertising and administrative expense. Since capital investment is responsible directly for depreciation and indirectly for other costs, it is important that a proper ratio (5:1) be maintained between yearly sales income and capital investment. Under present conditions, the capital investment should not exceed \$0.30/gal. of annual output.

Possibilities of reducing operating costs are limited. A plant producing its own mix should manufacture a gallon of ice cream with not to exceed 0.3 kw. of electricity, 1.5 lb. of steam and 22 gal. of water. Use of evaporative condensers and S.T.H.T. pasteurizers might result in a saving of 4.5 gal. of water/gal. of ice cream produced. The installation of automatic boilers has proved advantageous in many plants. A good inventory system is essential in reducing waste and conserving supplies. W. J. Caulfield

**436. Modalités et techniques de l'approvisionnement en lait des grands centres de consommation. (Modes and technics of supplying milk in large centers of consumption.)** Lait, 30, 291-292: 25-28. Jan.-Feb., 1950.

Certain problems relating to the distribution and consumption of pasteurized, evaporated and reconstituted dry milks in large population centers of France are discussed. S. Patton

Also see abs. no. 411, 412, 443.

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**437. Rumenal floral studies in the sheep. I. The nutritive value of rumen bacterial protein.** F. M. REED, R. J. MOIR and E. J. UNDERWOOD, Univ. of Western Australia, Nedlands. Australian J. Sci. Research, Series B, 2, 3: 304-317. 1949.

Rumen bacteria were separated from rumen fluid by differential centrifugation and then were dried in a hot air oven at 50-60° C. Proteins of dried rumen bacteria from sheep on "dry" feed, proteins of dried rumen bacteria from sheep on "green" feed and casein were compared in digestibility trials with rats. "True" digestibilities were found to be 64.8, 62.1 and 101.2 and biological values 77.9, 79.9 and 79.6, respectively, when fed at levels of 9.2, 9.7 and 9.5% crude protein. It is suggested that the value of micro-

bial protein might be improved by adding sulfur-containing amino acids. G. E. Stoddard

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

**438. Storage of bull spermatozoa at low temperatures.** AUDREY V. SMITH and C. PALGE, Natl. Institute for Medical Research, Mill Hill, N. W. 7. Vet. Record, 62, 9: 115-116. Mar. 4, 1950.

This study was designed to determine the effect of glycerol on the revival of bull and goat semen after freezing at very low temperatures. Motility alone was studied and no fertility trials were made. Glycerol was added to an egg yolk citrate diluent to give final concentrations of 5, 10, 15 and 20% glycerol. The diluted semen was cooled to -79° C. at different rates with a freezing mixture of solid CO<sub>2</sub> and alcohol. After varying periods the samples were thawed at +40° C. and compared with controls.

Ten per cent motility remained when glycerol-containing samples were frozen quickly at -79° C. and thawed after 5 min., while undiluted semen or diluted semen without glycerol showed no recovery. Revival increased when samples were cooled slowly, and were also greatest with higher glycerol concentration. Ninety per cent revivals were obtained with 15% glycerol cooled in 2.5-min. stages, with no difference noted between freezing time of 10 min. to 24 hr. Goat semen showed original motility following this treatment. R. P. Niedermeier

**439. The fecundity of the immature rat following induced superovulation.** C. R. AUSTIN, Natl. Inst. Med. Research, Hampstead, London, England. J. Endocrinol., 6, 3: 293-301. Jan., 1950.

Immature female rats, 30-45 d. old, each were injected subcutaneously with 20 international units (i. u.) of pregnant mare's serum, followed 56 hr. later by a subcutaneous injection of 20 i.u. of chorionic gonadotrophin. Following the second injection the females were placed with males. Dividing the rats into groups it was observed that 99% of the treated rats had ovulated. Only 34% of the treated rats had mated, however, and only 25% of the rats had fertilized eggs within 24 hr. after mating. The number of rats which showed implantation sites was 9%.

Although in these experiments superovulation was brought about with considerable success, the rate of fecundity was very low. Reasons for low fecundity were: (a) a failure to develop a complete state of estrus which led to a low incidence of mating and a poor transport of ova, (b) a low

rate of fertilization and (c) the shedding of abnormal eggs.

V. Hurst

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**440. The seasonality of calf births in England and Wales.** R. PHILLIPS, Univ. College of Wales, Aberystwyth. *Brit. Vet. J.*, **106**, 1: 18-29. Jan., 1950.

A statistical study is presented of calf births recorded in the quarterly returns of the Ministry of Agriculture for England and Wales for the period from June, 1944, to June, 1948. A discussion is given on the monthly percentage of calf births in England and Wales, average percentage monthly calf births, average percentage monthly calf births per county, month of conceptions, relationship of alternate quarters and the peak calving months. The annual distribution of calf births was March and October.

B. B. Morgan

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**441. Isothermal and isobaric degassing of ice cream.** A. LACHMANN, E. L. JACK and D. H. VOLMAN, Univ. of Cal., Davis. *Ind. Eng. Chem.*, **42**, 2: 391-394. Feb., 1950.

The quantities of air liberated from ice cream of normal composition have been measured in 2 series of experiments: (a) by lowering the applied pressure isothermally at selected temperatures between -10 and -50 ° C. and (b) by raising the temperature isobarically at selected pressures between 760 and 300 mm. The maximum quantity of air liberated was related to both the temperature and pressure to which ice cream of a definite composition was subjected. A decrease in pressure or an increase in temperature brought the ice cream to a point where the air cells were fractured and the amount of liberated air was considerably increased. The rate of diffusion of air through the ice cream changed at the temperature and pressure differential point where cell fracture occurred. It was suggested that the results could be applied to a study of shrinkage in ice cream and that a quantitative determination of lamellae strength might serve as a guide to possible shrinkage. B. H. Webb

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**442. Measurement of milk quality.** A. G. LEGGATT. *Can. Dairy Ice Cream J.*, **29**, 3: 86-88. 102. Mar., 1950.

A titration method for the estimation of the reducing capacity of milk has been developed. One ml. of a well-mixed sample is diluted with 10 ml. of distilled water and to this is added 1 drop of o-phenanthroline ferrous complex indicator (B.D.H. indicator). The sample is titrated to the discharge of the orange color by means of 0.01N ceric sulphate in N sulphuric acid solution from a micro burette. A pause of 30 sec., during which the orange color returns, is made and then more ceric sulphate solution is added until the same end-point is reached. The results of the titration may be expressed for convenience in milliliters of ceric sulphate. Greater values are obtained with abnormal milks.

II. Pyenson

**443. Transportvägens (-tidens) inverkan på mjölkens hållbarhet. (Transportation (time) as it affects the keeping quality of milk.)** T. BERGMAN and E. RAHMN. *Svenska Mejeriernas Riksförning Meddelande*, **6**: 1-8. Jan., 1948.

The centralization of the Swedish dairy manufacturing industry necessitates transporting milk over a greater distance. Three creameries (A, B and C) that received milk from long enough distances so that changes in the keeping quality of the milk during transit might be significant were chosen for study. The longest distance traveled was 8 Swedish (24 English) miles. Samples of milk were taken 3 times/mo., and about 98,000 samples were used for reductase tests during the investigations. An air-temperature record was made so that the effect of the temperature changes on the keeping quality of milk could be studied for every month of the year.

Long distance transportation tended to lower the quality of milk during the warm season of the year but not enough to be of concern to those who must depend upon long-distance transportation of their milk. G. H. Wilster

**444. "Baseball contest" spurs milk sales.** F. FLAGG. *Milk Plant Monthly*, **39**, 3: 52, 65. Mar., 1950.

To overcome the summer slump in sales, a baseball contest involving 10 six-man teams was created. With 2 teams playing against one another each week, the number of new quarts of business was the number of runs scored in each inning (weeks were divided into 7 innings). Weekly winners received \$30, whereas the winner of the "World Series" at the end of 10 wk. received \$200. J. A. Meiser, Jr.

**445. Incentives boost milk sales.** R. MILLER. *Milk Plant Monthly*, **39**, 3: 40-41. Mar., 1950.

To maintain routemen's enthusiasm at a high pitch and create sales when added business is especially desirable, routemen are paid \$1 for each point gained over their base period. The highest-scoring routeman also receives 50¢ for each point scored by the other men in the contest which gives this plan a double-barreled effectiveness.

J. A. Meiser, Jr.

Also see abs. no. 429, 430, 434, 436.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**446. The effects of thyroxine and thio-uracil on the secretion of the phosphorus compounds normally present in milk.** (Abs.) R. CHANDA and E. C. OWEN, Hannah Dairy Research Inst., Kirkhill, Ayr. *Biochem. J.*, **44**, 4: xxix. 1949.

When cows were injected with thyroxine, the ester and lipid P content of the milk increased while phosphatase decreased. The reverse was true when thio-uracil was given. A table shows the average P distribution in the milk from control, thyroxine and thio-uracil groups before, during and following treatment.

A. O. Call

## NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

**447. Factors influencing galactose utilization.** V. H. BARKI, P. FEIGELSON, R. A. COLLINS, E. B. HART and C. A. ELVEHJEM, Univ. of Wisconsin, Madison. *J. Biol. Chem.*, **181**, 2: 565-571. Dec., 1949.

Rats on whole milk diets excreted slightly less galactose than when on skim milk or skim milk plus cerelese diets. It is possible that the effect of the fat was to delay gastric emptying. The excretion of galactose was lower when an equivalent amount was fed in the form of lactose rather than as galactose. The major factor influencing the rate of galactose excretions seems to be the rate at which it reaches the circulation.

A. O. Call

**448. Significance of vitamin B<sub>2</sub> in milk diets.** (Abs.) R. A. COLLINS, L. S. DIETRICH and C. A. ELVEHJEM, Univ. of Wisconsin, Madison. *Feeder Proc.*, **9**, 1: 355. Mar., 1950.

Inferior growth in rats fed a mineralized goat's milk diet compared to good growth promoted by a similar cow's milk diet was investigated. The differences in growth were explainable on the basis of superior contents of folic acid and vitamin B<sub>12</sub> in cow's milk. Additions of vitamin

C to the cow's milk diet increased B<sub>12</sub> content of the livers, but showed no activity when added to the goat's milk diet.

S. Patton

**449. Recherches expérimentales sur la valeur antiscorbutique comparée des laits de vache frais, pasteurisés, bouillis, autoclavés, concentrés non sucrés, concentrés sucrés, secs non sucrés et secs sucrés.** (Studies of the vitamin C content of raw, pasteurized, boiled, autoclaved, unsweetened and sweetened condensed, and unsweetened and sweetened dry milks.) L. RANDOIN and A. PERROTEAU. *Lait*, **30**, 291-292: 29-32, 33. Jan.-Feb., 1950.

Vitamin C determinations were made on samples of milk and milk products using a protein precipitant of trichloroacetic-metaphosphoric acids reagent and measurement of vitamin C in the filtrate by use of methylene blue. Results are given for several samples of raw and pasteurized milk treated in various ways. Untreated raw milk was observed to vary in vitamin C content between 15 and 28 mg./l. Pasteurized (in bottle) milk gave values slightly lower than those of raw milk; however, such treatments as boiling and autoclaving lowered appreciably the vitamin C content of both. The effects of heat on the vitamin C content of milk is discussed. Concentrated and dry milks, whether sweetened or unsweetened, contained only insignificant quantities of vitamin C. It is recommended that fruit juice be fed in conjunction with milk and milk products in order to supply satisfactory quantities of vitamin C in the diet.

S. Patton

**450. L'acide pantothénique dans les produits laitiers.** (Pantothenic acid in dairy products.) A. HOUDINIÈRE. *Lait*, **30**, 291-292: 37-40. Jan.-Feb., 1950.

A review with 27 references.

S. Patton

**451. Recherches expérimentales sur le lait de vache actinisé. III. Influence de l'actinisation sur la teneur du lait en vitamine A.** (Investigations of irradiated milk. III. Influence of irradiation on the vitamin A content of milk.) J. BOISSELOT and J. CAUSERET. *Lait*, **30**, 291-292: 34-37. Jan.-Feb., 1950.

The object of the investigation was to study the effects of "actinisation," a rapid method of irradiation, on the vitamin A and carotene content of milk. Vitamin A was determined colorimetrically by the antimony trichloride method. Vitamin A and carotene were determined spectrophotometrically by absorption at 3280 Å. Total vitamin A activity was determined by bioassay. All three measurements indicated that

actinisation has little or no effect on the vitamin A content of milk. S. Patton

**452. Milk is still our finest food.** W. H. RIDDELL. Can. Dairy Ice Cream J., 29, 3: 82-84. Mar., 1950.

This is a general discussion of the food value of milk. H. Pyenson

**453. Amino acid content of evaporated milk on prolonged storage.** A. Z. HODSON, Pet Milk Co., Greenville, Ill. Ind. Eng. Chem., 42, 4: 694-695. April, 1950.

Samples of evaporated milk processed in the same plant but from different batches were analyzed for 17 amino acids. Fresh samples and samples that had been held in storage for 5 yr. were used. The amino acids were determined by microbiological methods. The only significant differences between the fresh and 5-yr.-old evaporated milks were losses of the aged samples in lysine (17%), histidine (17%) and arginine (11%). Analyses of 4 samples of evaporated milk held 15-17 mo. in storage indicated the losses of these 3 amino acids were not greater than 4% during this storage period. The losses in amino acids during the normal storage period for evaporated milk (less than 1 yr.) were considered nutritionally insignificant.

B. H. Webb

Also see abs. no. 427.

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**454. Improved intravenous technique in the large animals.** E. O. LONGLEY. Vet. Record, 62, 2: 15-16. Jan. 14, 1950.

Author describes a spear-pointed intravenous needle of hard stainless steel with a Record-type mounting used for both intravenous injections and bleeding. For intravenous injection an all-metal 2-way stop cock with male and female Record-type mountings is described and diagrammed which makes possible complete initial assembly of apparatus prior to injection and simple adjustment of rate of flow using either positive pressure or gravity feed. Name of London firm manufacturing this needle is given.

R. P. Niedermeier

**455. The sedimentation behavior of bovine and equine immune proteins.** E. L. SMITH and D. M. BROWN, Univ. of Utah College of Medicine,

Salt Lake City. J. Biol. Chem., 183, 1: 241-249. Mar., 1950.

Sedimentation behavior of immune proteins, previously isolated by chemical methods, was studied in the Spinco electrically-driven ultracentrifuge. The principal component of these immune proteins from the cow and the horse had a sedimentation constant of about 7 Svedberg units (defined as  $1 \times 10^{-13}$  sq. cm./sec., and abbreviated as *S*). Associated with it a second component of about 10 *S* was observed. Similar results were obtained with bovine  $\gamma$ -globulin prepared by the electrophoresis-convection method. The authors reemphasize earlier statements that the same components occur in the various bovine and equine fractions whether obtained from milk, colostrum or plasma. H. J. Peppler

**456. Partial specific volumes for some porcine and bovine plasma protein fractions.** V. L. KOENIG, Upsala Univ., Sweden. Arch. Biochem., 25, 2: 241-245. Feb., 1950.

Bovine and porcine plasma protein fractions, prepared according to Cohn's procedure and previously examined ultracentrifugally, were employed in the determination of the apparent partial specific volume as indicated by the pycnometer technique of Drucker. The following average values of partial specific volumes were obtained for bovine fractions: fibrinogen, 0.706;  $\gamma$ -globulin, 0.725;  $\beta$ -globulin, 0.714;  $\alpha$ -globulin, 0.722; crystalline albumin, 0.730. Average values for the porcine fractions were 0.744 ( $\gamma$ -globulin), 0.766 ( $\beta$ -globulin) and 0.731 for the impure albumin. The high values of porcine  $\beta$ -globulin may be due to traces of lipids. These values for bovine and porcine plasma protein fractions differ from those reported for human fractions.

H. J. Peppler

**457. Studies on proteins from bovine colostrum. III. The homologous and heterologous transfer of ingested protein to the blood stream of the young animal.** R. G. HANSEN and P. H. PHILLIPS, Univ. of Wisconsin, Madison. J. Biol. Chem., 179, 2: 523-27. June, 1949.

Colostrums from the cow, goat and pig, taken immediately following parturition, were fed to new-born goats. Electrophoretic studies were made on the kids' blood before and after ingestion of each colostrum, and in each case there was an increase in "immune" proteins, but varying in degree for each source. These "immune" proteins seem to pass unchanged from the colostrum to the blood stream.

New-born calves were divided into 3 groups before nursing their dams. The control group



was fasted; a 2nd group was given mid-lactation milk; and the 3rd was fed cow colostrum. The serum of all calves at birth, showed the presence of proteins immunologically similar to "immune" proteins from colostrum; the amount, however, was increased by feeding milk and still further by feeding colostrum.

A. O. Call

**458. Molybdenum metabolism and interrelationships with copper and phosphorus.** C. L. COMAR, L. SINGER and G. K. DAVIS, Florida Agr. Expt. Sta., Gainesville. *J. Biol. Chem.*, **180**, 2: 913-922. Sept., 1949.

It was shown that in rats a high Mo intake retarded growth, but when accompanied by a high Cu intake growth was equal to groups on low Mo and low Cu; hence Mo toxicity must be considered in terms of the Cu level. Radioactive  $\text{Mo}^{90}$  and  $\text{P}^{32}$  were used to study their metabolism in the steer. Tables are given showing the tissue distribution of Mo and P when fed orally to 1 steer and when administered by jugular injection to another. A similarity between the behavior of these 2 elements in the bovine is pointed out. Radioisotopes of Mo, Cu and P were used in studying their interrelationships in the rat. Several probable mechanisms for Mo toxicity are postulated.

A. O. Call

Also see abs. no. 425.

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**459. The sanitarian and the milking machine.** T. R. ENRIGHT, Klenzade Products, Inc., Beloit, Wis. *Milk Plant Monthly*, **39**, 2: 68-70. Feb., 1950.

Prior to establishing an efficient, uniform procedure for cleaning and storing milking machines, accumulated deposits of milkstone must be removed by immersing all of the milker parts in a solution of 1 part organic acid cleaner and 4 parts warm water for 20 min., followed by vigorous brushing. A daily procedure that will maintain a physically clean machine is as follows: (a) flush equipment after milking with tepid water ( $100^{\circ}\text{F.}$ ); (b) dismantle machine and brush wash all parts in warm water ( $125^{\circ}\text{F.}$ ) to which an alkaline cleaner has been added; (c) every 4 d. substitute a balanced organic acid cleaner for the alkaline type in the brush washing procedure; (d) rinse the scrubbed parts in hot water ( $145^{\circ}\text{F.}$ ); (e) invert parts on metal drying racks and (f) prior to using, sanitize the assembled equipment with a liquid sodium hypochlorite solution. Wet storage solutions for rubber milking parts should be prepared using an organic acid detergent rather than lye or chlorine, since the latter compounds cause the rubber to become porous, thus preventing proper cleaning.

J. A. Meiser, Jr.

Also see abs. no. 408.

# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

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### BOOK REVIEW

**460. Elements of Dairying.** 2nd ed. T. M. OLSON. The Macmillan Co., New York, N. Y. 708 pp. 1950.

This book, originally printed in 1938, is designed to serve as a text for use of college students in a first course in dairying. In the revised edition, the material is discussed under the three main headings of dairy cattle, dairy products and dairy farming. Needed new information dealing with the selection of individual dairy cattle, maintaining a profitable herd, a cropping system for dairy farms, prevention and care of diseases affecting dairy cattle, nutrition deficiencies, dairy farm buildings (including equipment) and plans for acquiring a dairy farm have been added by the author. In the appendices new material has been added, including a list and history of the dairy organizations and up-to-date score cards on dairy products and dairy cattle. The tables dealing with nutrition of dairy cows have been shortened with some elimination and other tables covering growth of dairy cattle have been added. At the end of each chapter there are questions on the important points covered. The book is well organized, illustrated and indexed. C. Y. Cannon

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**461. The treatment of chronic bovine mastitis with aureomycin.** R. A. PACKER, Iowa State College, Ames. Vet. Med., **45**, 5: 199-201. May, 1950.

Aureomycin was used for treatment of 91 quarters with chronic staphylococcal infections. Except for 1 animal, all of the animals treated were from 2 herds. Diagnosis of the infection was made by isolation and identification of the causative organism. Of 70 quarters infected with *S. aureus* treated with 1 200-mg. dose of aureomycin hydrochloride, 34.3% were considered freed of the organisms. *S. aureus* was eliminated in 68.5% of 35 quarters by the administration of 2 injections of aureomycin. The drug had little

or no effect on 2 cases of *E. coli* infection or 12 cases of chronic streptococcal mastitis. The aureomycin was incorporated in an ointment base and dispensed in collapsible tubes. The drug was injected from these tubes directly in the teat canal after the regular milking period. The conclusions were that aureomycin is of definite value in the treatment of chronic bovine staphylococcal mastitis. B. B. Morgan

**462. Report on subtilin and bacitracin as possible treatment for bovine mastitis.** J. O. HEISHMAN, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, **11**, 39: 206-210. Apr., 1950.

The bactericidal action of subtilin and bacitracin was tested against several strains each of *Str. agalactiae*, *Str. uberis* and hemolytic staphylococci on blood agar plates. *Str. agalactiae* was the most resistant to these antibiotics. A combination of bacitracin and penicillin was more effective than either one alone in preventing growth of these 3 groups of organisms. The *in vitro* tests indicated that these antibiotics should be effective against such organisms in mastitis cases. Infusions of subtilin, bacitracin and penicillin-bacitracin combination both in single and double doses were made into udders of cows known to be infected. Improvement in appearance of milk and clinical condition followed, and the udder tolerated the substances without noticeable disturbance of milk production. No significant permanent elimination of infection occurred, however, so these antibiotics were judged to be no better than others now generally available. E. W. Swanson

**463. Pathogenesis of bovine mastitis. II. The pathologic alterations in twenty-five glands.** G. R. SPENCER and S. H. McNUTT. Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **11**, 39: 188-198. Apr., 1950.

Examination of the gross and microscopic changes produced by mastitis infection in 18 udders from herds in which complete antemortem history and examination were available and 7

udders without antemortem information was made. Typical cases were described in detail. Nineteen of 43 quarters with no clinical history of mastitis were eliminating streptococci, and 13 of these showed varying areas of focal mastitis. More detailed examination may have revealed similar conditions in the remaining 6. Of 24 quarters having neither clinical nor bacteriological evidence of mastitis, 4 had foci of inflammation similar to those found in streptococcal mastitis. Palpation was found to be a poor method of diagnosing fibrosis. Firm areas in the gland were most often interstitial edema and retained secretions. Atrophy and fibrosis were most often found together. The inflammatory foci were characterized by a blocking of the small ducts with fibrin, leucocytes and organisms with a consequent distension of the alveoli with secretion and edema in the interstitial tissues. Following stasis of secretion, large numbers of organisms developed in the milk areas and the epithelium was destroyed. The principal site of the inflammatory changes was the ventral portion of the gland. Long standing cases had extensive areas of atrophy and fibrosis with a thickening and roughening of the large duct and cistern walls. These changes were readily detectable by gross inspection of the dissected gland. The indications were that inflammation was caused by and accompanied infection and aided the progress of infection by hindering the normal milk flow. A difference in susceptibility of cows is postulated on the basis of the wide variation in pathological changes which were poorly correlated with the duration of infection. E. W. Swanson

**464. The *Brucella abortus* ring test.** M. H. ROEPKE, K. G. PATERSON, F. C. DRIVER, L. B. CLAUSEN, L. OLSON and J. E. WENTWORTH. Minn. Agr. Expt. Sta., St. Paul. Am. J. Vet., Research, 11, 39: 199-205. Apr., 1950.

The ring test, conducted by mixing 1 drop of stained *Br. abortus* antigen with 1 ml. of milk and noting after about 1 hr. the amount of the dye in the cream layer and skimmilk layers, has been widely used in Denmark in a brucellosis control program. The application of the test in Minn. herds under an area control plan was investigated in 9 counties. Since most of the herds marketed cream, an adaptation of the test for cream samples was developed. Most of the tests were taken before the country-wide blood tests. The blood and ring tests agreed in 96.2% of 8,469 herds. The ring test was 68% efficient in locating infected herds; however, 65% of the infected herds not located did not have an infected cow in production so the ring test was 88% efficient for infected herds in which the infected animals were

producing. False positives from the ring test were attributed to contamination of the milk weighing vat, frozen milk and imperfect technique. The test is proposed as a helpful adjunct to the blood test on the area control plan. E. W. Swanson

**465. Persistence of *Brucella abortus* infection in cattle.** C. A. MANTHEI and R. W. CARTER, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, 11, 39: 173-180. Apr., 1950.

Presence of *Br. abortus* infection was detected by inoculation of guinea pigs and by cultural means in several groups of naturally and artificially infected cows over periods of 2 yr. Bacteremia was compared in unvaccinated, strain 19-vaccinated and strain 45/20-vaccinated cows. It was lowest in strain 19-vaccinated and highest in strain 45/20-vaccinated. Peak bacteremia was reached at 2 wk. Highest levels of bacteremia were accompanied by high abortion rate and high persistence of the infection. Groups of 24 naturally-infected and 38 artificially-infected cows were followed for successive pregnancies up to the 9th. Eight animals in each group ceased shedding *Br. abortus*. Recoveries of *Br. abortus* from uterine material, colostrum and blood and the number of abortions revealed no detectable difference between the course of natural and artificially produced infections. One group of 18 cows was artificially infected with a virulent strain of *Br. abortus*. The distribution of the organism was followed therein for 2 pregnancies, followed by autopsy of 15 cows and examination of the lymph glands for *Br. abortus*. Udder infection occurred in 17 of these cows and persisted in 16. The supramammary lymph gland was the commonest site of infection. Genital infection was erratic but was most persistent in a repeat-breeder cow. *Br. abortus* was found in 1 cow for 97 wk. and in another for 101 wk. *Br. abortus* was not found in spleens, livers, kidneys, ovaries, vaginas, bile, urine or mesenteric or iliocecal lymph glands from this group of cows. E. W. Swanson

**466. Q fever studies in southern California. IX. Isolation of Q fever organisms from parturient placentas of naturally infected dairy cows.** L. LUOTO and R. J. HUEBNER, Nat'l. Inst. of Health, Bethesda, Md. Pub. Health Reports, 65, 16: 541-544. Apr. 21, 1950.

The authors tested placental tissue of 33 serologically positive cows and found that 13 (39%) contained *C. burnetii*. Some placental tissues were infectious for guinea pigs after diluting as high as 1-100,000,000. They also found that *C. burnetii* was encountered more often during first parturitions than in subsequent ones.

The authors were unable to demonstrate the presence of *C. burnetii* in the placentas of 4 serologically negative cows. D. D. Deane

**467. Anthrax in livestock during 1949 and incidence of the disease from 1945 to 1949.** C. D. STEIN, B. A. I., Washington, D. C. Vet. Med., 45, 5: 205-208. May, 1950.

A survey made in 1949 showed that 93 anthrax outbreaks were reported from 16 states with a loss of 773 animals. The outbreaks were sporadic and occurred primarily in cattle. Of the 93 outbreaks, 56 occurred in California, Louisiana and Texas. During the 5-yr. period from 1945-1949, 597 outbreaks involving 7,909 livestock in 32 states were reported. Abattoirs under Federal meat inspection during this 5-yr. period condemned 38 cattle for anthrax. Twenty cases of anthrax occurred in man, 15 in agricultural workers and 5 in veterinarians. The conclusions were that anthrax was of considerable importance and that every effort should be made to prevent its occurrence.

B. B. Morgan

**468. Bovine endometritis—A review of literature to 1947, with special reference to the catarrhal type of the disease.** F. L. M. DAWSON, Ministry of Agriculture and Fisheries, Weybridge, England. Brit. Vet. J., 106, 3: 104-106. Mar., 1950.

A brief chronological review of the literature on bovine endometritis is given. The dates of the periodicals consulted ranged from 1843 to 1947. The paper is divided into several sections: (a) first controversial period: 1900-1924, (b) rise of "Nielsenism": 1925-1935, (c) discovery of reproductive hormones: 1935 onward, (d) bacteriological aspects and (e) clinical research methods.

B. B. Morgan

**469. Studies with johnin and tuberculin intradermal tests in cattle naturally infected with *Mycobacterium paratuberculosis* (Johne's disease).** D. SIKES and A. H. GROTH. Louisiana State Univ., Baton Rouge. Am. J. Vet. Research, 11, 39: 181-187. Apr., 1950.

Intradermal injections of johnin and tuberculin were made at 6-mo. intervals from 1940-1944 in a herd of 400 cattle. A summary of the Johne's disease-positive cattle showed that reactions to johnin from the candall fold site were not as sensitive as from a previously unused neck site, being 9.5 and 77.6%, respectively. Reading at 48 hr. gave about 50% more positive responses than reading at 72 hr. Although the herd was presumed to be free of tuberculosis, 1.8% gave positive reactions to tuberculin at the candall fold site

and 11.8% gave positive reactions at the neck site. The sensitivity of Johne's disease-infected cattle to tuberculin persisted for many months.

E. W. Swanson

Also see abs. no. 482.

## BUTTER

O. F. HUNZIKER, SECTION EDITOR

**470. Report on trials of the Alfa buttermaking process.** G. L. HILLS, L. BALLARD, F. WILKINSON, M. THOMAS and L. R. HUNTER, Australian Dairy Produce Board, Melbourne. (Mimeoprint) 1949 (?).

This preliminary report covers a number of trials in which the Alfa process of continuous buttermaking is compared with conventional churning (in wooden churns) by using divided lots of the same creams under controlled conditions in an Australian butter factory. The average score of the freshly made butter was practically the same for both processes, but occasionally tallowy flavors would develop in the Alfa butter due to Cu contamination from accessory equipment. The microbiological quality of the Alfa butter was superior to that of the churned butter, as shown by consistently lower total counts and almost complete absence of yeast and coliform contamination. The moisture in the Alfa butter was better dispersed than in the churned butter. Moisture distribution and other physical properties of the Alfa butter could be controlled by adjustment of the brine temperatures in the transmutator unit. The Alfa butter was shiny in appearance and had better spreadability at low temperature than churned butter. It also showed greater tendency to oil off when standing at 86° F. for 3 hr.

Composition control in the Alfa process is accomplished by the continuous addition of salt and water (or skim milk); under good conditions water and salt contents could be adjusted within  $\pm 0.10\%$  of the desired figure. The Alfa process shows an advantage in overrun of 1.1% (based on fat loss and composition), but a cost study indicated that its manufacturing cost was 0.3-0.4¢/lb. than that of churned butter. However, this finding was distorted by the fact that the Alfa unit was not operated at full capacity.

The Alfa process as applied in this study is suitable only for sweet cream, since neutralized cream would cause excessive sludge deposition in the separator and interfere with the composition control. Other aspects of practicability and operation of the equipment are discussed.

V. H. Nielsen

**471. Comparison of several methods for determining the butterfat content of sour cream.** C. B.

LANE and R. L. FRANCE, Breakstone Bros., Inc. Laboratories, Walton, N. Y. Milk Plant Monthly 39, 4: 38-39, 71. Apr., 1950.

Sour cream was tested by the Babcock, Mojonnier and Roese-Gottlieb methods. Samples were obtained from vat pasteurizers after homogenization and standardization had been completed. The Babcock procedure used 30%, 18 g., sealed, long-necked, 0.2% graduated bottles, into which 18 g. of sample were weighed. Fourteen to 17 ml. of sulfuric acid were added in 3 portions, after which 5-10 ml. of water at 60° C. were added. Centrifuging and reading of the tempered samples were according to the recommended Babcock testing procedure for cream. An average of the results obtained for 106 trials showed the Babcock procedure to be 0.30% below the Mojonnier method and 0.09% lower than the Roese-Gottlieb method. The Roese-Gottlieb method averaged 0.21% lower than the Mojonnier method.

J. A. Meiser, Jr.

## CHEESE

A. C. DAHLBERG, SECTION EDITOR

472. **A new type of bacterial spoilage in Canadian process cheese.** E. G. HOOD and J. F. BOWEN, Science Service, Dept. of Agr., Ottawa. Sci. Agr., 30, 1: 38-42. Jan., 1950.

Two widely separated outbreaks of bacterial spoilage of process cheese food were investigated. The defective packages were badly swelled, contained gas holes and possessed a very obnoxious, putrefactive odor. Non-fat dry milk solids were used in the blends and the defect became apparent in samples held at 100° F. for 1-4 d. or in retail stores at summer temperatures in 1-2 wk.

An organism corresponding closely to *Clostridium sporogenes* was found to be responsible and was present in half the samples of non-fat dry milk solids used in the cheese food blends. Experimental batches made up with varying amounts of non-fat dry milk solids, with 2% casein digest and with 1-yr.-old cheese developed the defect when inoculated with the organism and incubated 5 d. at 98° F.

O. R. Irvine

Also see abs. no. 484, 486, 496.

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

473. **Browning and the fluorescence of evaporated milk.** N. P. TARASSUK and H. D. SIMONSON, Div. of Dairy Ind., Univ. of Cal., Davis. Food Technol., 4, 3: 88-92. 1950.

A method was developed for measuring the

fluorescence of evaporated milk after the proteins were digested with pancreatin. A marked increase in fluorescence occurs during heat sterilization of evaporated milk, and the chemical changes responsible for the increase in fluorescence continue during storage. Browning and fluorescence develop simultaneously during sterilization of evaporated milk, and their formation proceeds at a parallel rate, but the browning and fluorescent materials are not necessarily identical. Both browning and fluorescence show a close relationship to CO<sub>2</sub> production. The data indicate that the fluorescing and browning materials are associated with the milk protein and become soluble only when the protein is hydrolyzed. Fluorescence studies denote that high-temperature, short-time sterilization will reduce the brown discoloration and cooked flavor of evaporated milk and thereby produce a superior quality product.

E. R. Garrison

474. **The "browning reaction" in dried milk powder.** J. B. MOSTER and R. A. CHAPMAN, Macdonald College, Quebec. Can. J. Research, Sec. F, 27, 11: 429-434. Nov., 1949.

Heated and stored dried whole milk powders showed a marked loss of amino nitrogen, as determined by the Van Slyke volumetric method, when compared with fresh powder. No such loss was observed when the formol titration was used. Titration curves (from pH 6.0-11.0) of the powders suggested a mechanism for the protein-sugar condensation. The heating of synthetic mixtures of amino acids and lactose resulted in intense browning, accompanied by a loss of amino nitrogen when a large excess of lactose was employed (1:13), but no loss occurred when equal parts of sugar and amino acid were present.

O. R. Irvine

475. **Mechanical cow lowers milk price in Whitehorse.** L. HARRINGTON. Food in Canada, 9, 12: 26, 28, 30. Dec., 1949.

Details are given of the reconstituting process by which non-fat dry milk solids, butter and water are combined to make a 4.2% fat milk. Sixty-five lb. of water, 6.25 lb. of powder and 3.25 lb. of butter are mixed at 100-112° F., after which the batch is pasteurized at 145° F. for 30 min. It then is homogenized, cooled and packaged in paraffined containers. Introduction of the product resulted in a lowering of the price of milk from 85-30¢/qt. in this Yukon community.

O. R. Irvine

476. **Special milk powders for manufacture of milk chocolate.** H. A. HOLLENDER (Abstract of a thesis for Doctor of Philosophy degree at the

University of Wisconsin). K. E. LANGWILL. Confectioner J., 75: 44-47. Nov., 1949.

This thesis treats the subject of milk lipolysis and its effect on "milk" flavor of milk chocolate. Milk powders were prepared under varying temperatures and time treatment relationships with percentages of sugar from 0-10%. Powders then were incorporated into an experimental milk chocolate formula, heated and ground in a mortar to a plastic mass containing no visible particles of sugar or milk powders. Relation of free fatty acid to "milk" flavor was observed at 15 intervals. "Milk" flavor has a definite relationship to free fatty acids. Milk chocolate having the most desirable flavor has the highest free fatty acid content and the lowest pH. The lipase activity of the raw whole milk powders is accelerated by increased temperature of storage. This phenomena is not observed in milk chocolate containing milk powders prepared from properly forewarmed milk. Milk powders prepared with sugar seem to be conducive to best "milk" flavor. Tables are given to substantiate results. T. A. Eggers

477. *Preparacion y conservacion de la mezcla lactea Escudero con leche acidofila y leche bifida.* (Preparation and preservation of Escudero's milk mixture with acidophilus and bifidus milk.) S. SORIANO and A. M. DE SORIANO. Rev. Asoc. argentina dietol., 6, 23: 235-242. July, Aug., Sept., 1948.

A study was made of the keeping quality of Escudero's milk mixture (cereal water, milk, lactose, cream) used for feeding children. This mixture, when submitted to summer room temperatures (25° C.), became unacceptable within 24 hr. because of its high bacterial content. Increasing the acidity to 3.5% by the addition of lactic acid reduced the microflora development, and the product was acceptable for 48 hr. Acidification could be accomplished by adding lactic acid or milk fermented with *Lactobacillus acidophilus* or *L. bifidus*. An initial acidity as high as 3.5% was not necessary if the milk mixture was sterilized before inoculation with the pure cultures. The use of pure cultures had the disadvantage of requiring a bacteriologist. L. S. Olsen

478. *Concentrated milk food product and process of preparing same.* W. H. HOECKER and B. W. HAMMER. (Assignors to Golden State Co.) U. S. Patent 2,501,445. 8 claims. March 21, 1950. Official Gaz. U. S. Pat. Office, 632, 3: 871. 1950.

The fat/milk solids-not-fat ratio of milk is adjusted to 0.5-1.75/1 by the addition of butterfat. After concentrating to 40-70% total solids and

pasteurizing in the range of 150-190° F. for 0.5-30 min., the product is homogenized while hot at a pressure in the range of 500-3500 lb./in.<sup>2</sup>. This product is free of cooked flavor, keeps well at refrigerator temperatures, is easily spreadable, has a uniform and smooth texture and is resistant to changes in viscosity at temperatures encountered during storage and use. R. Whitaker

479. *De mogelijkheid van het verwerken van weibloem in brood.* (On the possibility of using whey flour in the baking of bread.) (English summary.) H. HEERES and E. A. M. MEYKNECHT, State Dairy Organization, The Hague, Holland. Neth. Milk and Dairy J., 4, 1: 54-79. Jan.-Mar., 1950.

A number of experiments in a commercial bakery were made to test the possibility of using whey flour in the baking of bread. Substitution of part of the wheat flour by whey flour caused a smaller loaf volume; in using 2%, a volume decrease of 2.7% was obtained which could not yet be considered significant. Baking flour consisted of: I. 80% of wheat flour of foreign origin + 20% of flour of home grown wheat. II. 90% of mixture I + 10% of potato flour. In case II with 2% whey flour, 4.2% decrease in volume was found. This tendency to diminish the volume of the loaf was the only objection to the use of 2% whey flour, as other properties remained the same or were slightly better. Difference in processing or fat content of the whey gave the same result. Lactose, a lactalbumin preparation, casein and dried skim milk (roller and spray) all caused a greater decrease in volume. Use of 2% whey flour in bread would take away a good part of the whey surplus. It would be good from a nutritional standpoint, as minerals and vitamins would be supplied which are deficient in plain normal bread. A. F. Tamsma

480. *Concentration of albumin from whey.* G. J. STREZYNSKI. (Assignor to DeLaval Separator Co.) U. S. Patent 2,500,101. 6 claims. March 7, 1950. Official Gaz. U. S. Pat. Office, 632, 1: 289. 1950.

Whey is fed into the bowl of a centrifuge where an albumin-rich portion collects on the peripheral wall and a lactose-rich, albumin-free portion is removed from the central area. The albumin-rich portion, containing some lactose, leaves the bowl through ports, is collected and diluted with water and is directed back into the bowl through a channel ending at the ports. By proper balancing of the diluted albumin against the whey intake, a lactose-free albumin can be recovered from the ports. R. Whitaker

**481. Method of stabilizing dried starch sirup.** T. Nordenskjöld and E. A. Jönsson. U. S. Patent, 2,501,406. 1 claim. March 21, 1950. Official Gaz. U. S. Pat. Office, **632**, 3: 860. 1950.

To produce a dehydrated hydrolyzed starch product, free from hygroscopicity, the sirup is spray dried with milk. R. Whitaker

Also see abs. 503.

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**482. Effects on acid production by lactic starters of various "drugs" in milk from mastitis-treated cows.** W. A. KRIENKE, Fla. Agr. Expt. Sta., Gainesville. Milk Plant Monthly, **39**, 4: 32, 36-37. Apr., 1950.

The addition of 5.0 ml. of a 25% solution of sulfamethazine to 100 ml. of "drug-free" milk almost completely inhibited acid production when inoculated with 1% active buttermilk culture. One ml. and 0.1 ml. additions of the "drug" resulted in a developed acidity of 0.35% and 0.46%, respectively, as compared to 0.71% for the control. Thus, milk from 1 treated cow would render the milk from more than 80 untreated cows unfit for fermented dairy products.

The addition of 0.005 mg. of aureomycin hydrochloride to 1 ml. of "drug-free" milk greatly retarded the production of lactic acid in starters. However, reducing the concentration to 0.00005 mg. allowed a nearly normal acid production. A single infusion of 200 mg. of aureomycin hydrochloride thus would inhibit acid production in 1,000 lb. milk and retard it greatly in 1,400 lb. milk.

Milk from aureomycin-treated cows contained sufficient amounts of the "drug" after 12 milkings to retard acid production considerably. When 1% of the milk from the 1st milking was combined with 99% of the "drug-free" milk, acid production was completely inhibited. Mixtures containing 10% of milk from treated cows and 90% of milk from untreated cows did not favor acid production until the 6th milking.

The use of penicillinase as an inactivator of penicillin in milk was not practical since the cost of the enzyme necessary to permit normal acid development exceeded the cost of the milk.

J. A. Meiser, Jr.

**483. Effect of concentration and reaction (pH) on the germicidal activity of chloramine-T.** G. R. WEBER, Pub. Health Service, Cincinnati, O. Pub. Health Reports, **65**, 15: 503-512. Apr. 14, 1950.

The pH of a chloramine-T compound greatly

affects its germicidal activity. Those compounds with a pH higher than approx. 7.5 were found to be too slow for practical use where short exposure periods were used. Increasing the concentration of the chloramine-T compound from 50-1,500 ppm. did not reduce the killing time sufficiently to equal that of even the more alkaline hypochlorites at 50 ppm. concentration. A chloramine-T compound in a concentration of 250 ppm. with a pH of not more than 7.0 or a concentration of 500-1,000 ppm. at a pH of not over 7.5 appeared to have a germicidal action, in the absence of organic matter, as rapid as that of the slower (alkaline) hypochlorites at 50 ppm. The author reported that commercial chloramine-T products do not as a rule have a pH as low as 7.0-7.5 and concluded that, while chloramine-T compounds appear to have a limited value where rapid germicidal action is needed, they may be the sterilizer of choice under conditions where long exposure periods are necessary. D. D. Deane

**484. Syrningsvanskeligheder forårsaget af Bacteriophage. (Starter failures due to bacteriophage.)** A. J. OVERBY, Danish Royal Vet. & Agr. College Dairy Laboratory. Mælkeritidende, **62**, 47-48. 1949.

For the first time, bacteriophage active against multiple-strain starters has been demonstrated in Denmark. The phage did not survive a temperature of 85° C. (185.0° F.) for 5 min. or a temperature of 88° C. (190.4° F.) for several seconds. The bacteriophage was active against 11 of 14 isolated single cultures of lactic streptococci from "slow" starter.

One strain of the bacteriophage did not pass through a Seitz filter. Two electron micrographs showed that the head of the bacteriophage had a diameter of 0.15 $\mu$  and the tail had a length of 0.30 $\mu$ .

In a creamery that had experienced difficulty with slow starter, it was thought that bacteriophage from the air entered the starter and cream-ripening vats. After thoroughly sanitizing all equipment and atomizing a 5-10% hypochlorite solution in the various manufacturing rooms, no more difficulty was experienced.

The literature review contains 51 references. G. W. Wilster

**485. Estimation of lipase in dairy products. II. An extraction-titration method for the estimation of bacterial lipase.** D. J. LUBERT, L. M. SMITH and H. R. THORNTON, Univ. of Alberta. Can. J. Research, Sec. F, **27**, 12: 491-498. Dec., 1949.

In testing for bacterial lipase a loopful of the culture is inoculated into 10 ml. of sterile skim

milk and incubated for 24-48 hr. The lipase then is estimated on 2 ml. of this culture using a procedure similar to that of abstract 494. Ether-soluble acids carried into the reaction medium do not interfere with the measurements and ether-soluble acids are not produced from protein or lactose during the test. The main test organism was *P. fluorescens*.  
O. R. Irvine

**486. Estimation of lipase in dairy products. III. Lipase activity in cultures of micro-organisms and in cheese.** D. J. LUBERT, L. M. SMITH and H. R. THORNTON. Can. J. Research, Sec. F, 27, 12: 499-503. Dec., 1949.

The lipolytic activity of a number of micro-organisms was determined by the method of abstract 485. No organism produced a bacterial lipase having an activity optimum on the acid side of neutrality. No lipase activity at approx. pH 5.0 was demonstrated in 20 samples of commercial cheddar cheese or in 1 sample of blue veined cheese by this method, or by the method of Peterson *et al.* (J. Dairy Sci., 31, 1: 31-38. 1948). Weak lipolytic activity was found in 1 sample of blue cheese by the extraction-titration method. One sample of cheddar displayed no lipolytic activity when tested at pH 8.50.

O. R. Irvine

**487. Estimation of lipase in dairy products. IV. Lipolytic activity of *Pseudomonas fluorescens*.** D. J. LUBERT, L. M. SMITH and H. R. THORNTON. Can. J. Research, Sec. F, 27, 12: 504-509. Dec., 1949.

Lipolytic activity of a strain of *P. fluorescens* was greatest when the reaction medium was at approx. pH 8.9 at the start of a reaction period and when the reaction was carried out at approx. 42° C. The lipase hydrolyzes tricaproin and tricaprolyn less readily than tributyrin.  $\text{CaCl}_2$  inhibited activity. Lipolytic activity was greater in nutrient broth-base medium than in skim milk, but the former gelled when ether was added. Lipolytic activity and fluorescence were not related.

O. R. Irvine

**488. Preservation of foods with antibiotics. I. The complimentary action of subtilin and mild heat.** A. A. ANDERSEN and H. D. MICHENER. Western Regional Research Lab., Albany 6, Cal. Food Technol., 4, 5: 188-189. May, 1950.

This new principle in food preservation is based on the destruction of enzymes and microorganisms with subtilin and mild heat. Some of the non-spore-forming bacteria, particularly the Gram-negative ones, are resistant to subtilin but sensitive to heat, while the heat-resistant organisms, such

as clostridia and thermophiles, are extremely sensitive to subtilin with mild heat. Peas, asparagus, corn, green beans, peeled potatoes, tomato juice and milk have been preserved from microbial spoilage by this method of treatment. Experiments with peas, asparagus and corn were described to illustrate the process and its effectiveness in food preservation. In general, the addition of 10 or 20 ppm. of subtilin prevented spoilage when these foods were sealed in no. 1 cans, and the cans heated in boiling water for 10 or 20 min. and then stored at 77 and 122° F. All of the control cans (without subtilin but heated) spoiled during storage. The possible physiological effects of continued use of foods containing subtilin and other antibiotics has not been determined, and additional information on this subject is needed before safe use can be made of this principle in preserving foods.  
E. R. Garrison

**489. Contribucion al conocimiento de las bacterias lipoliticas de la manteca. (Contribution to the identification of the lipolytic bacteria of fat.)** A. M. SORIANO. Rev. Asoc. Argentina dietol., 6, 24. 284-292. Oct., Nov., Dec., 1948.

One hundred and three samples of fats acquired in the stores in the city of Buenos Aires were studied microbiologically with respect to the content of lipolytic bacteria capable of producing rancidity. The bacteria were isolated using Turner's differential plating medium for lipase-producing bacteria and the bacteria then were identified. Of the samples examined, 52.4% were contaminated with lipolytic bacteria belonging to 8 species, 5 of which belonged to the genus *Pseudomonas*. Fifteen samples contained no lipolytic bacteria.

The species encountered and the frequency with which they occurred in the samples examined were: *Pseudomonas traslucida* 33.85%, *Ps. incognita* 30.18%, *Ps. fluorescens* 28.30%, *Ps. arguata* 6.14%, *Ps. mira* 3.07%, *Bacillus effusus* 15%, *Achromobacter superficiale* 1.88% and *Achr. fomesum* 1.88%.  
L. S. Olsen

**490. Salt tolerance in the genus *Aerobacter*.** I. O. FODA and R. H. VAUGHN, Div. of Food Technology, Univ. of Cal., Berkeley. Food Technol., 4, 5: 182-188. May, 1950.

Fifty-two cultures of coliform bacteria were isolated from olive brines by direct plating on Levine's E.M.B. agar after enrichment of the brines in glucose broth containing 10% salt. These cultures were identified as *Aerobacter aerogenes*, but differed from the common types of this species in their appearance on E.M.B. agar and from all other coliform bacteria tested because of their striking resistance to NaCl. In-



creased tolerance to salt, which extended up to 14.5% NaCl with some cultures, was obtained by periodically transferring the cultures to glucose broth with increasing salt concentrations. The additional resistance gained through acclimatization was adaptive and was readily lost when the bacteria were returned to a salt-free environment.

E. R. Garrison

**491. Partial purification of a factor essential for growth of *Leuconostoc citrovorum*.** J. C. KERESZTESY and M. SILVERMAN, Nat. Institute of Health, Bethesda, Md. J. Biol. Chem., **183**, 2: 473-479. Apr., 1950.

Concentration of an acid-labile factor required for the growth of *Leuconostoc citrovorum* (ATCC 8081) was achieved by norit adsorption of liver extract (fraction S) and butanol extraction of the concentrated eluates. In media lacking folic acid the concentrates containing the citrovorum factor promoted the growth of *Streptococcus lactis* R (*S. faecalis* R) and *Lactobacillus casei*. The citrovorum factor stored at room temperature for 24 hr. in 0.1N HCl lost 90-100% of its activity for *L. citrovorum*, but only 41-48% of its activity for *S. lactis* R. A similar treatment of folic acid failed to alter its activity. The implication of these results are discussed; however, further purification of the citrovorum factor is necessary before a satisfactory interpretation can be offered.

H. J. Pepper

**492. Vitamin B<sub>12</sub> and "citrovorum factor" in the nutrition of *Lactobacillus leichmannii* and *Leuconostoc citrovorum*.** T. H. JUKES, H. P. BROQUIST and E. L. R. STOKSTAD, Lederle Lab., Pearl River, N. Y. Arch. Biochem., **26**, 1: 157-159. Mar., 1950.

Chromatographic and cultural studies provided an indication that the "citrovorum factor" (CF) is a compound which contains folic acid. The attending observations further suggest that certain precursors are converted to B<sub>12</sub> (reaction A), which in turn participates in the conversion of other precursors into the desoxyribosides of guanine, adenine, hypoxanthine and cytosine. Also, folic acid is converted to CF (reaction B), which in turn participates in the reversible conversion of thymidine to the desoxyribosides. Previous findings established that vitamin B<sub>12</sub> or the desoxyribosides of either guanine, hypoxanthine, adenine, cytosine or thymine promote the growth of *Lactobacillus leichmannii* 313, while CF or thymidine, but not the other desoxyribosides or vitamin B<sub>12</sub>, permitted the growth of *Leuconostoc citrovorum* 8081 in a purified culture medium. Thus, *L. leichmannii* may accomplish step B in the above scheme, but not step A, while *L. citro-*

*vorum* would be able to carry out step A but not step B. The scheme is given further support by the discovery that *L. citrovorum* produced vitamin B<sub>12</sub> activity in purified media, and *L. leichmannii* synthesized CF.

H. J. Pepper

**493. Utilization of optical isomers of methionine and formylmethionine by some lactobacilli.** J. R. SPIES and D. C. CHAMBERS, U.S.D.A., Washington, D. C. J. Biol. Chem., **183**, 2: 709-712. Apr., 1950.

The relative degrees of utilization of pure optical isomers of methionine and formylmethionine in a defined medium by *Lactobacillus arabinosus* 17-5, *Leuconostoc mesenteroides* P-60 and *Streptococcus faecalis* R were determined by an acidimetric method. None of the bacteria utilized D-methionine or formyl-D-methionine at levels of 6γ or 10γ/ml. medium. Only *S. faecalis* utilized L-methionine and its formyl derivative; growth with the latter was slightly better than it was with free L-methionine. Pyridoxine was found ineffective in promoting the utilization of D-methionine by *L. arabinosus*.

H. J. Pepper

Also see abs. no. 461, 462, 472.

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**494. Estimation of lipase in dairy products. I. An extraction-titration method for the estimation of milk lipase.** L. M. SMITH, D. J. LUBERT and H. R. THORNTON, Univ. of Alberta. Can. J. Research, Sec. F, **27**, 12: 483-490. Dec., 1949.

Milk lipase is determined by allowing 2 ml. of the skim milk to react on 0.6 ml. of tributyrin for 30 min. at 37° C. at pH 8.8 in the presence of borate buffer. The reaction is stopped by adding phosphoric acid and reducing the temperature, after which the reaction medium is extracted with ethyl ether. An aliquot of the ether layer then is titrated. The result of a blank determination is deducted from this value. Such factors as extraction efficiency, substrate concentration, pH, temperature and length of reaction period were examined and are discussed.

O. R. Irvine

**495. Detecting alien fats.** W. H. MARTIN, W. D. RITZ and C. H. WHITNAH, Kansas State College, Manhattan. Ice Cream Field, **58**, 6: 20, 23, 24, 57, 58, 59, 61. Dec., 1949.

See abs. 75.

**496. New and improved methods of extracting fat from cheese, fresh curd and milk for fat acidity determination.** J. F. BOWEN, E. G. HOOD

and C. A. GIBSON, Dominion Dept. of Agr., Ottawa. Sci. Agr., 29, 11: 551-552. Nov., 1949.

To secure samples of fat from cheddar cheese, approx. 250 g. are ground in a Waring blender and heated in the dry state on a boiling water bath until "oiled off." In separating fat from fresh curd, approx. 800 g. are finely ground in suitably-sized portions in the blender. To each portion 500 ml. of 90° C. water is added and thoroughly mixed, after which all portions are combined and held at 0° C. until a fat layer has formed. This fat layer then is churned, clarified by centrifuging and filtered. Samples of fat from milk are obtained by churning the fat, after which it is melted, centrifuged and filtered. The "acid degree" of these fat samples then is determined by titrating a 10-g. portion in boiling 95% neutral ethanol with 0.1 N NaOH, using phenolphthalein as indicator.

O. R. Irvine

**497. Rate of destruction of reduced ascorbic acid in riboflavin-fortified pasteurized milk.** A. D. HOLMES. Mass. Agr. Expt. Sta., Amherst. Food Technol., 4, 3: 92-93. 1950.

The milk used in this study was produced by a 70-cow herd composed of 5 breeds and was pasteurized at 143° F. for 30 min. in stainless steel vats. Riboflavin was added to 19 weekly samples of the freshly pasteurized milk in amounts of 0.0, 4.0 and 8.0 mg./l., and the milk stored in the dark at 10° C. Ascorbic acid determinations were made on the samples after 0, 24, 48, 72, and 96 hr. of storage. Additions of riboflavin did not increase rate of loss of reduced ascorbic acid in pasteurized milk. Samples fortified with 0, 4 and 8 mg. of riboflavin/l. showed an average loss of 77, 73 and 69%, respectively, of the original amounts of reduced ascorbic acid after 96 hr. of storage.

E. R. Garrison

**498. Effects of borate and other ions on the alkaline phosphatase of bovine milk and intestinal mucosa.** C. A. ZITTE and E. S. DELLA MONICA, Eastern Regional Research Lab., Philadelphia, Pa. Arch. Biochem., 26, 1: 112-122. Mar., 1950.

The inhibitory effect of borate and other anions on alkaline phosphatase prepared from cow's milk and calf intestinal mucosa was studied in ethanolamine-HCl buffer containing sodium phenylphosphate; the phenol liberated was determined with the reagent of Follin and Ciocalteu. Both milk and mucosa phosphatases were inhibited competitively by sodium tetraborate, apparently of the anionic type, while the inhibition of milk phosphatase by ethanolamine was found to be of the noncompetitive (cationic) type. Milk phosphatase closely resembles kidney and bone phosphatases and is distinguished from

the intestinal mucosa enzyme by its higher pH optimum, lower enzyme-substrate constant ( $K_s$ ) at pH 9.6, greater inhibition by cations and lesser interference by anions. The inhibitory effects of the anions phosphate, pyrophosphate, carbonate and arsenate on the alkaline phosphatases are given for comparison with the data obtained with tetraborate.

H. J. Peppler

**499. Effects of glutamic acid, lysine and certain inorganic ions on bovine alkaline phosphatases.** C. A. ZITTE and E. S. DELLA MONICA, Eastern Regional Research Lab., Philadelphia, Pa. Arch. Biochem., 26, 1: 135-143. Mar., 1950.

Earlier studies (*ibid.*, 26, 1: 112-122) of attempts to distinguish between 2 types of alkaline phosphatases by determining the relative effects of anions and cations have been extended to include observations on the effects of lysine, glutamic acid, carbonate and the ammonium ion. Milk phosphatase was inhibited to a greater extent by lysine and the ammonium ion than was the intestinal mucosa phosphatase; the latter enzyme was inhibited more strongly by glutamic acid and carbonate ion. Low substrate concentrations of lysine stimulated milk phosphatase. The results further the suggestion that there are 2 types of alkaline phosphatases, the intestinal enzyme and the milk enzyme, the latter appearing to be similar to the phosphatases of bone and kidney.

H. J. Peppler

**500. The properties of the enzyme-substrate compounds of lactoperoxidase.** BRITTON CHANCE, Medical Nobel Inst., Stockholm. J. Am. Chem. Soc., 72, 4: 1577-1583. Apr., 1950.

Although the mechanisms of action of lactoperoxidase and horse-radish peroxidase appear to be identical, the oxidations of the milk enzyme proceed at a much faster rate than those of the plant enzyme. In spite of the differences between their hemes and proteins, both enzymes exhibit similarities in the formation of primary peroxide complexes, alkyl hydrogen peroxides and the oxidation of pyrogallol and ascorbic acid.

H. J. Peppler

Also see abs. no. 471, 473, 474, 523.

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**501. Waste prevention in the dairy industry.** Task Committee on Dairy Waste Disposal. Milk Dealer, 39, 6: 51-52, 104-106. Mar., 1950.

The more common causes and methods of eliminating excessive waste losses in dairy plants are: (a) Leakage and drippage, such as the constant and continual loss of milk from improperly

assembled or fitted equipment. (b) Overflow, which can be greatly reduced if not completely eliminated by careful attention and by the use of liquid level control devices. (c) Spillage, largely due to careless handling. (d) Freezing-on, which can be minimized with adequate refrigerant controls and proper operation. (e) Willful waste. Perhaps the largest volume of milk solids entering the drainage system is put there more or less willfully or get there because no effort is made to save them. (f) Residual waste, the total losses from which may reach amazing proportions unless care is taken to allow time for proper drainage. (g) Separators, the open type of which produces large quantities of foam, causing loss of milk solids.

C. J. Babcock

**502. Waste prevention in the dairy industry.** Task Committee on Dairy Waste Disposal. *Milk Dealer*, 39, 7: 47-48, 56-63. Apr., 1950.

The simplest device for measuring the flow of waste is a standard 90° V-notch sharp-crested weir located in a weir box and equipped with either a hook gauge or water level recorder. Instructions and detailed drawings for the construction and use of a weir are given. C. J. Babcock

**503. Method of drying protein.** E. ERICKSON. (Assignor to Hercules Powder Co.) U. S. Patent 2,502,134. 2 claims. March 28, 1950. *Official Gaz. U. S. Pat. Office*, 632, 4: 1166. 1950.

Casein or other protein is dried on a perforated belt, by passing heated air countercurrently through the belt in a series of tunnel compartments. The moisture-laden air is reheated before entering the 1st compartment to cause the curd to adhere to the belt on its immediate entrance to the drying tunnel.

R. Whitaker

**504. A new approach to plant planning.** G. R. JOHNSON, Pace Associates, Chicago, Ill. *Ice Cream Rev.*, 39, 9: 48, 56, 60. Apr., 1950.

Success in the dairy processing field demands the use of a plant and facilities designed to meet, (a) present production requirements, (b) probable future expansion of the business without seriously disrupting operations and (c) high standards of efficiency and flexibility of operation.

Formulation of any expansion program should be based upon careful study and analyses of all factors involved. The probable cost of expanding an existing plant and facilities should be carefully weighed against the cost of a new plant. In making such comparisons, maintenance and operational costs should be carefully studied in addition to the initial investment. In 1 instance cited, savings of \$20,000 in plant costs and

\$75,000 in operational costs over a 10-yr. period would have resulted from building a new plant rather than enlarging the old one.

A careful study of all factors will enable the plant owner embarking on a building program to do so with confidence, for his decisions will be based upon facts and not guesswork.

W. J. Caulfield

**505. Automatic ventilation of common storages.** J. H. L. TRUSCOTT, E. W. FRANKLIN and JOY GILLIAT, Ontario Agr. College, Guelph. *Sci. Agr.*, 29, 11: 497-511. Nov., 1949.

Equipment is illustrated and described which has performed satisfactorily in maintaining uniform temperatures in unrefrigerated storages during the period Oct. 4-Apr. 19, at levels of 32 and 40° F. The storage rooms are equipped with constantly-running fans to ensure air circulation within the rooms. Cooling is accomplished by drawing air through a duct at floor level past an automatic shutter. Warmer air is expelled from the room at ceiling level past an automatic shutter by a fan connected to a differential thermostat and operates when the outside air temperature is below that within the storage. This fan also is cut off thermostatically when the room temperature falls to the desired level. A thermostatically controlled heat source also is connected to the air circulation system and may be used if necessary. The specially-designed differential thermostat is described and the performance of the equipment is related to weather records for the district.

O. R. Irvine

Also see abs. no. 470.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**506. Receiving milk by tanker pick-up system.** H. G. MOJONNIER, Mojonnier Bros. Co., Chicago, Ill. *Milk Dealer*, 39, 6: 47, 107-108. Mar., 1950.

Edisto Farms, Dairy of Columbia, S. C., has inaugurated a tanker pick-up system which promises to improve quality and at the same time bring other labor and product saving advantages inherent in bulk handling methods. The milk is cooled to 38° F. in stainless steel insulated refrigerated producer's tanks on the farm. A milk pump with piping for transferring the milk is carried on the tanker. At each farm the amount of milk in the producer's tank is ascertained by measuring the depth of the milk with a stainless steel ruled measuring stick. At 2 farms the pick-up is made only every other day but there

is no significant difference in bacterial count as a result of 2 d. holding on the farm. A charge of 20¢/cwt. is made for the tanker pick-up service. As the milk is transported in bulk in a well insulated tank there is only about 1° F. rise in temperature during transportation. The system is a labor saver as the handling of both full and empty cans is eliminated.\* C. J. Babcock

**507. Lowering costs through efficient plant management.** H. A. RUEHE, Univ. of Ill., Urbana. *Milk Plant Monthly*, 39, 4: 64, 66-67, 74. Apr., 1950.

Efficient plant management requires a continuous inspection of the following business phases: (a) physical plant, (b) procurement, (c) processing and (d) sales. Sound judgement based on the above findings is the difference between a reasonable profit and complete failure.

J. A. Meiser, Jr.

**508. Know your profit per line.** F. MERISH. *Milk Plant Monthly*, 39, 4: 52-54, 56. Apr., 1950.

Indirect expenses, which include office expenses, advertising, delivery and other administrative or commercial outlay, must be prorated to the various lines in a plant if a company wishes to determine the profit or loss per line. Advantages of this system are: (a) provides experience figures for setting up prices, (b) determines if sales volume per line is ample to cover overhead, (c) gauges efficiency of operations and (d) obtains a true picture of yearly profits.

J. A. Meiser, Jr.

**509. Automatic sales accounting.** E. D. PAULSON, Menzie Dairy Co., McKeesport, Pa. *Milk Dealer*, 39, 7: 44-45, 64-66. Apr., 1950.

The advantages of punched card accounting system are: (a) Reports are more easily obtained on time. (b) Special reports can be prepared more easily through the use of the punched cards, because once the information is recorded in punched form, a variety of reports can be printed other than the routine ones. (c) Routemen are relieved for more productive work since the machines do their "paper work." (d) Reports are automatically printed by machines and, since they are on a standard form, they are much easier to read. (e) More comprehensive reports may be obtained just as easily as all routine reports and without additional expense. (f) It has reduced the cost of forms. (g) Due to the flexibility of equipment used, other applications also may be performed. The farmer's payroll also is prepared. This includes checks, check registers and other product reports. C. J. Babcock

**510. Boosting sales of by-products.** T. KNIGHT. *Milk Plant Monthly*, 39, 4: 92-93. Apr., 1950.

Each product handled by the plant was rated according to the point system. Utilizing the previous month's sales as the base period, each routeman was given a base number for each by-product sold. The object of the contest was to meet this quota or better it. For men who sold 60-70% of their quota, an award of \$2.50/mo. was given. Those reaching 90% or better received \$5.00/mo. To insure added sales, the quota was changed each month, taking into account the seasonal demand for individual by-products. In addition to the above plan, incentive programs were incorporated to promote milk sales and reduce route returns. This latter contest paid cash bonuses of \$5.00 and \$10.00 for the leading routemen.

J. A. Meiser, Jr.

**511. Outlook for ice cream and dairy products.** R. C. HIBBEN, IAICM, Washington, D. C. *Ice Cream Trade J.*, 46, 5: 56. May, 1950.

The weather, buying power, quality of product and merchandising effort are factors which will influence ice cream sales in 1950. For the first time in its history the majority of ice cream manufacturers engaged in a merchandising program concentrating their sales effort on a single flavor, cherry-vanilla.

The fluid milk industry is faced with a problem of disposing of an increased supply of milk resulting from a record production. Intensified sales training programs, now engaged in by many milk companies, are producing results.

Increased production of butter will necessitate greater sales effort. Such programs now are under way. Prospects are for increased imports of cheese and decreased exports; this may result in lower prices for cheese. The dry milk industry is faced with the problem of doubling domestic sales; failure to do so may result in a demoralized market. The outlook for the evaporated milk industry for 1950 is for a stabilized demand throughout the year.

Also see abs. no. 501, 504.

W. H. Martin

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**512. Stability of carotene in alfalfa meal. Effect of antioxidants.** C. R. THOMPSON. Western Regional Research Laboratory, Albany, Cal. *Ind. Eng. Chem.*, 42, 5: 922-925. May, 1950.

The conditions of exposure of finely divided alfalfa meal to oxygen are severe and most edible antioxidants lack sufficient activity to afford the

necessary protection. Structure of the compound appeared to be correlated with antioxidant activity. 2,5-disubstituted hydroquinones, p-substituted phenylenediamines, and derivatives of 2,2,4-trimethyl-1,2-dihydroquinoline were the most active compounds tested. Vegetable oils plus acetone were suitable solvents. The addition of increasing amounts of antioxidants gave increased stability but approached a limit above which additional amounts gave no effect. B. H. Webb

**513. Pastures studies XXIX and XXX. Investigations on the lignin fractions of pasture herbage and of the feces of ruminants. I. The lignin fraction of pasture herbage. II. The lignin fraction of the feces of ruminants.** F. J. SOWDEN and W. A. DELONG, Macdonald College, Quebec. *Sci. Agr.*, 29, 9: 409-417, 418-423. Sept., 1949.

Finely ground samples of herbage collected at several periods during 1942 and 1943 were air dried and finely ground. Lignin then was determined by the standard (Manning-DeLong) and Crampton-Maynard methods. The results indicated that widely different amounts of lignin were isolated and that the fractions differed in purity, as indicated by nitrogen and methoxyl content. Absorption spectra on 3 samples of forage lignin when compared to that of wood lignin confirmed the presence of impurities in both types of fractions. The ratio of clover to grasses in immature herbage may influence the nature of the fractions isolated.

Samples of the above herbage were fed to a steer in 1942 and to sheep in 1943 and samples of the feces were collected and analysed for lignin. Oven drying resulted in higher apparent lignin content in the isolates than did freezing and extracting before drying. Lignin content on the 1942 samples isolated by the standard method ranged from 14.85-16.32% and by the C-M method from 23.92-26.69%. Nitrogen and methoxyl values showed the C-M fractions to be less pure. Spectrographic analysis and solubility values in sulphite solution indicated that both fractions were about 50% pure relative to wood lignin. The data, however, suggest that lignin is not demethoxylated in its passage through the animal.

The study reveals the need for more accurate methods of lignin analysis before this means can be used as an accurate index of digestibility of herbage. O. R. Irvine

**514. Roller crusher for drying hay.** J. W. WHITE and W. KALBFLEISCH, Experimental Farms Service, Ottawa. *Sci. Agr.*, 30, 3: 119-124. Mar., 1950.

A crusher consisting of 2 spring-loaded steel rolls, 6 in. in diam. and 5 ft. long and driven by

an auxiliary 45 h.p. engine is used to hasten the drying of hay in the swath. On early-cut hay, drying time was reduced from 2-3 d. to 1 d. by crushing. O. R. Irvine

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

**515. Examination of bull semen with the ordinary and phase contrast microscopes.** P. G. D. MORRIS, Royal (Dick) Veterinary College, Edinburgh, Scotland. *Brit. Vet. J.*, 106, 3: 85-93. Mar., 1950.

Observations were made on the semen of bulls with the ordinary and phase contrast microscopes. Using the phase microscope reduced the risk of artefacts which may appear in stained preparations. An attempt was made to classify certain morphological variations of the anterior portion of the head beneath the galea capitis as seen with the phase microscope. Three types were classified: (a) sperm with a dark zone under the anterior portion of the membrane of the galea capitis and separated from the nuclear substance by a narrow light zone, (b) sperm showing a diffuse grey zone below the anterior portion of the limiting membrane and (c) sperm showing a clear zone between the membrane of the galea capitis and the extremity of the nucleus. Ten good photomicrographs illustrate the paper.

B. B. Morgan

**516. The effect of homogenization, pasteurization and lyophilization on egg yolk-sodium citrate diluents for bull semen.** J. B. HERRICK, Iowa State College, Ames. *Am. J. Vet. Research*, 11, 39: 159-160. Apr., 1950.

Egg yolk for diluting semen was prepared by homogenizing followed by lyophilizing egg yolk, sodium citrate (3%) and sulfanilamide (0.3%). This mixture was reconstituted at the rate of 3 parts yolk to 5 parts distilled water and filtered through cheese cloth. Survival time of bull sperm in the reconstituted egg yolk diluent was equal to that in diluent made with fresh egg yolk. Refrigeration of the lyophilized product for 30 d. at 40° F. was without effect. Pasteurization of the prepared diluent was not harmful and produced a product which could be stored refrigerated without development of contamination and without precipitation of yolk material.

E. W. Swanson

**517. A study of size inheritance in the house mouse. I. The effect of milk source.** L. BUTLER and J. D. METRAKOS, McGill Univ. *Can. J. Research*, Sec. D. 28, 1: 16-34. Feb., 1950.

Three strains of mice were used to study the effect of fostering on the growth pattern of the mouse. The strains used breed true for size and have been designated as "large," "small" and "intermediate." The 14-d. mean weight of mice that received milk from "large" strain mothers is significantly different from those that received milk from either the "small" or the "intermediate" strain mothers. Although these differences tend to remain, they are not statistically significant at 140 d. The significance of these results are discussed in relation to the arithmetic and geometric concepts of polygenic growth. O. R. Irvine

## HERD MANAGEMENT

### II. A. HERMAN, SECTION EDITOR

**518. Suckling device for calf feeders.** P. O. STEVENS. (Assignor to Mutual Products Co.) U. S. Patent 2,501,146. 9 claims. March 21, 1950. Official Gaz. U. S. Pat. Office, **632**, 3: 790. 1950.

A pail of liquid calf feed, held in a slightly tilted position by a rack, is provided with a tube leading from the lowest corner of the pail to a nipple held in a horizontal position over the rack. Suction provided by the suckling calf draws the feed from the pail to the nipple. R. Whitaker

**519. Cattle stanchion.** H. A. DUMFORD. U. S. Patent 2,499,819. 15 claims. March 7, 1950. Official Gaz. U. S. Pat. Office, **632**, 1: 217. 1950.

A U-shaped stanchion is hinged on the bottom and attached to the floor by means of a chain; the top is attached to a bar but is arranged for easy opening and closing. The bar is attached to an upper support or to the ceiling by means of a centrally located swivel which permits rotary movement of the stanchion. R. Whitaker

## ICE CREAM

### C. D. DAHLE, SECTION EDITOR

**520. The suspending power and viscosity of carrageenin.** R. C. ROSE and W. H. COOK, Natl. Research Laboratories, Ottawa. Can. J. Research, Sec. F, **27**, 9: 323-336. Sept., 1949.

Commercial and laboratory prepared samples of carrageenin were heated in milk at 70° C. for 20 min., cooled rapidly to 10° C. and stored at 5-10° C. for 24 hr., when viscosity determinations were made. Suspending power varied from sample to sample but was closely related to viscosity ( $r=0.98$ ). The high viscosity of cold milk containing as little as 0.04% carrageenin appears to be due to the formation of a casein-carrageenin gel which is heat sensitive. Viscosity-concentration curves for whole milk and skim milk were al-

most identical. That for dialysed milk was similar. The behavior of carrageenin in 0-0.5 N solutions of NaCl, CaCl<sub>2</sub> and KCl also was studied. The correlation coefficient between suspending power in milk and viscosity of 0.05 N NaCl was 0.91, suggesting that the latter could be used to predict the former. O. R. Irvine

**521. Seaweed extracts as a food thickening.** R. C. ROSE, Natl. Research Laboratories, Ottawa, Can. Food in Canada, **9**, 11: 9-11. Nov., 1949.

Agar, sodium alginate and carrageenin are food and beverage thickeners derived from 3 types of seaweeds. Canada has ample quantities of alginate- and carrageenin-bearing seaweeds which are harvested along the coasts of the Maritime provinces.

Carrageenin is a hot water extract of *Chondrus crispus*, known commonly as Irish moss or carrageen. The extract is filtered, concentrated and dried. It thickens foods by gelling and by reacting with milk protein. The addition of potassium salts increases the gelling temperature of the solution and the strength of the resulting gel. The stabilizing effect of small amounts of carrageenin in chocolate milk recently has been shown to be due to a gelling action on the milk proteins.

O. R. Irvine

**522. Stabilizers and emulsifiers in ice cream.** F. E. POTTER and D. H. WILLIAMS, U.S.D.A., Washington, D. C. Ice Cream Rev., **33**, 9: 148-151. Apr., 1950.

Stabilizers aid in producing smooth texture in ice cream hydration, formation of a gel structure throughout the mix or reaction with certain milk constituents to form substances that take up water as water of hydration. In selecting a stabilizer, ease of incorporation into the mix, effect on mix viscosity, type of body produced in ice cream, ability of the stabilizer to retard ice crystal growth, quantity required to stabilize the mix and cost must be considered. Pertinent data with respect to 12 different stabilizing agents for ice cream are summarized by the authors.

Emulsifiers are ester combinations of long-chain fatty acids with a higher alcohol, such as glycerol or sorbitol. Emulsifiers may be classified into 3 groups which are: (a) a mixture of mono-glycerides and diglycerides, (b) esters of fatty acids and sorbitol or other higher alcohols and (c) polyoxyalkylene derivatives of group b. The chemical structure of each group of emulsifiers is presented. Emulsifiers aid in promoting dispersion of the fat. They tend to orient themselves at the fat-water interface in the mix, thereby reducing interfacial surface tension and retarding clumping of fat globules. Emulsifiers do not re-

place stabilizers but provide a supplementary effect which results in a drier ice cream and possibly a smoother texture. Use of emulsifiers in ice cream has not been ruled on as yet by regulatory officials.

W. J. Caulfield

**523. Shrinkage of ice cream as affected by the state of milk proteins.** N. P. TARASSUK and J. T. HUTTON, Univ. of Cal., Davis. *Ice Cream Trade J.*, 46, 5: 44. May, 1950.

Shrinkage was determined by subjecting pint samples of ice cream, which had been stored at  $-10^{\circ}$  F. for 48-72 hr. and tempered at  $2^{\circ}$  F. for 3 d., to a vacuum of 230 mm. of mercury for 2 min. and then replacing them in the cabinet at  $2^{\circ}$  F. for 5 d.; the volume of water required to fill the space evacuated by the ice cream was determined. Surface tension, viscosity, pH, titratable acidity and protein stability were determined, the latter by the temperature of coagulation on addition of 0.30 ml. of 2%  $\text{CaCl}_2$  to a 5-ml. portion of mix.

A modified Hull spectrophotometric test for tyrosine was used to determine the effect of incipient hydrolysis of proteins of the mix on shrinkage of ice cream. The higher concentration of milk solids in ice cream, as compared to milk, necessitated the addition of 20 ml. of 0.72 *N* trichloroacetic acid, in place of 10 ml. for the precipitation of proteins. Upon addition of the phenol reagent, the filtrate becomes cloudy and requires refiltration before making spectrophotometric color determinations. About 80% of the blue color developed in the test was attributed to tyrosine and 20% to tryptophane. Results were expressed in "tyrosine units." A unit is 1 mg. of tyrosine/l. of sample, or its equivalent.

A direct relationship between shrinkage and overrun throughout the range of 90-130%, was found to exist. Ice cream containing emulsifiers of the Span and Tween series contained smaller air cells and was more susceptible to shrinkage. Addition of diglycol laurate, a surface tension lowering agent, resulted in increased shrinkage due to the presence of free fatty acids. No correlation was found between the use of previously frozen mix ingredients (cream and condensed skim milk) and shrinkage.

Shrinkage susceptibility is markedly influenced by differences in milk from individual cows, possibly due to inherent breed characteristics. Wide differences in pH and protein stability observed in individual milks could not be correlated with shrinkage. However, the correlation between shrinkage and tyrosine value is outstanding. Aging of mixes resulted in a definite and consistent increase in shrinkage.

The addition to the mix of a hydrolysate pre-

pared from acid-precipitated casein at the rate of 0.1% (calculated as dry unhydrolyzed casein) and the mix allowed to stand overnight increased shrinkage. These tests also indicated that products other than tyrosine were responsible for shrinkage.

Heat denaturation of lactalbumin and globulin were studied. As the heat treatment of the mix was raised, shrinkage increased. Whey proteins added to the mix to replace the proteins precipitated by heat markedly decreased shrinkage. The undenatured globulin fraction of whey protein was a factor in reducing shrinkage; addition of lactalbumin appears to increase shrinkage.

W. H. Martin

**524. Soft ice cream and your business.** W. A. JOSEPHSON, Sou. Dairies, Inc., Birmingham, Ala. *Ice Cream Field*, 55, 3: 74-76. Mar., 1950.

A too "rich" and "eggy" flavor, coupled with poor sanitation, are the reasons given for discontinued success of the old "custard" type soft ice cream.

The new soft ice cream industry is credited with starting in southern Illinois and northern Missouri and has made rapid gains on the Pacific Coast. The so-called soft ice cream machines are based on the principle of extruding ice cream, continuously or intermittently, at a temperature of about  $16-19^{\circ}$  F.

A survey in Los Angeles County, Cal., reported that 36-40% of the ice cream sold is soft. Soft ice cream outlets in the county, on the average, sold 5 times the gallonage per store as did the competing conventional outlets. Owners of these stores are drawn from nearly all walks of life and the patrons represent a cross section of American life.

The success of these stores is due to guidance from franchise and equipment people in getting started. Beyond that they depend on the following: (a) Soft ice cream is good. (b) Soft ice cream usually is a low-fat, high-solids product which is not too rich. (c) The value of low overrun is recognized, 50-55% usually being taken. (d) These stores generally are operated under sanitary conditions. (e) They nearly always employ the drive-in principle. (f) The operation is kept simple and as a result is profitable.

Soft ice cream in California, it is felt, has decreased the sale of hard ice cream, whereas in other localities this effect is not so prominent. The ice cream industry should consider better merchandizing methods, improved sanitation, as well as the possible use of so-called "converts" which will convert small portions of hard ice cream into soft ice cream, or still another con-

verter which will extrude soft ice cream from a can of hard ice cream in a cabinet. W. C. Cole

**525. A pint of sundaes.** Anonymous. Ice Cream Trade J., 46, 5: 30, 32, 68. May, 1950.

A new type of combination package including a pint of ice cream and a transparent plastic "bag" of sundae topping in 1 convenient carton has been introduced by a number of ice cream manufacturers in the midwestern markets. The plastic bag which contains the proper amount of topping for 4 or 5 servings withstands subzero temperatures. Sponsors of the combination package believe that convenience, economy and the desire of the consumer for sundaes will result in increased sales of ice cream.

W. H. Martin

**526. Pre-cut ice cream cakes on "production line" basis.** Anonymous. Ice Cream Trade J., 46, 3: 64-65. Mar., 1950.

Redi-kut ice cream cake is made in a special 2.5-qt. mold consisting of 20 individual segments. The segmented mold may be filled direct from the freezer. The top of the mold then is clamped down. In the top of the mold and separate from it is a disk holding 20 metal spikes which become imbedded in the ice cream. After the mold containing the ice cream is passed through a brine tank, it is plunged into hot water, the top of the mold is removed and the segments of the cake adhere to the disc containing the spikes. The segments then are pushed by a lower movable disc onto the base of a cardboard cake-dispensing unit. A steel ring brings the segments together and a 0.5-in.-high flexible card-board strip stapled to 1 edge of the cake-dispensing unit is closed with a clasp holding the cake in shape for decorating. The decorated cake is ready for delivery or storage in the hardening room.

W. H. Martin

**527. Ice cream bars go 'round.** A. K. VELAN, Velan Eng. Co., Montreal, Can. Ice Cream Field, 55, 3: 78-79. Mar., 1950.

A description is given of a rotating machine designed to automatically freeze, chocolate coat and wrap ice cream bars. It was developed in Denmark and Switzerland and is known as the RIA system.

W. C. Cole

**528. Ice cream cup.** A. A. HEYMAN. (Assignor to Maryland Baking Co.) U. S. Patent 2,501,939. 4 claims. March 28, 1950. Official Gaz. U. S. Pat. Office, 632, 4: 1117. 1950.

A crisp pastry cup with tapering sides for nesting and a flared top portion having an internal notched ring to provide an anchorage for the ball of ice cream is described.

R. Whitaker

**529. Package-filling spout for ice cream machines.** R. J. H. LANE. U. S. Patent, 2,502,329. 1 claim. March 28, 1950. Official Gaz. U. S. Pat. Office, 632, 4: 1216. 1950.

This device, easily attached to an ice cream freezer, has a sliding valve which, when lifted, allows the ice cream to pass through a suitably shaped opening into the package.

R. Whitaker

**530. Ice cream.** C. F. KOERVER. (Assignor to the Borden Co.) U. S. Patent 2,500,315. 6 claims. March 14, 1950. Official Gaz. U. S. Pat. Office, 632, 2: 457. 1950.

The ratio of lactose to mineral salts normally present in ice cream mix is increased 10% by addition of lactose to improve the flavor, impart an additional refreshing sensation when eaten and overcome "slickness," especially in high-fat ice cream.

R. Whitaker

**531. Frozen purees from citrus fruits.** E. A. BEVENS, Bur. of Agr. and Ind. Chem., Pasadena, Cal. Ice Cream Field, 58, 6: 26, 62, 63. Dec., 1949.

Experiments conducted by the Laboratory of Fruit and Vegetable Chemistry in Los Angeles in 1947 and since show that satisfactory frozen citrus purees can be prepared. Sound, mature fruit is washed with a good detergent and then rinsed well with cold water. Next, the stem end is cut off and other dark specks are removed; in the case of Navel oranges the "navel" end should be cut off. Next, the fruit is quartered or crushed and finally reduced to a puree by passage thru a mechanically driven screwing device with minimum incorporation of air. Screen sizes of 0.027 and 0.033 are preferable when purees are intended for use in sherbets, ices, pies and beverages, but larger sizes are better where the purees are to be used for marmalades, jams or toppings.

The yield of puree from whole fruit is about 50-60%; 0.65-0.75% peel oil is recommended. To control the oil content it may be necessary to pass part of the fruit thru an abrasive machine before it is quartered or crushed in order to remove most of the oil sacs.

One part of sugar is added to 5 parts of puree. This mixture then is placed in containers and the contents frozen in an air blast at sub-zero temperatures. The containers are stored at 0-10° F. Lacquered or enameled cans are recommended for high-acid purees. Purees can be kept satisfactorily for more than a year.

Navel orange purees can be stored for several months without bitter flavors developing but upon prolonged storage, the purees gel. This problem now is being studied.



Orange and lemon purees have been used successfully in commercial milk sherbets and water ices. Sherbets with 2.5% butterfat were considered better than water ices. It is recommended that 14-18 oz. of 5:1 orange puree and 1.5 oz. of citric acid (50% solution) be added/gal. of sherbet mix. W. C. Cole

**532. Report on apple ice cream.** J. C. HENING and C. S. PEDERSON. N. Y. State Agr. Expt. Sta., Geneva, N. Y. *Ice Cream Field*, 55, 4: 62, 64, 65. Apr., 1950.

A new type apple juice and the use of apple juice concentrate in ice cream is reported. Ice cream made with this concentrate appeared like vanilla ice cream but had a strong true apple flavor. The success of apple ice cream depends upon the preparation of the juice and concentrate.

McIntosh apple juice was prepared by the ascorbic acid method of Pederson (1947) and Holgata, *et al.* (1948). The concentrate was prepared by the freezing concentration described by Pederson and Beattie (1947). The ascorbic acid inhibits the action of oxidizing enzymes during extraction, deaeration and pasteurization. Pasteurization was accomplished at 165-175° F. for 20 sec., with cooling in 30-lb. enamel-lined cans. Concentration to 3.6:1 was accomplished by slow freezing to the desired degree and then removing the ice by centrifuging. McIntosh apples will yield 60-65% juice it is claimed.

This McIntosh juice concentrate was used in ice cream to the extent of 24%. Baldwin concentrate blended with McIntosh 1:4 gave a good product but other concentrates were too acid. McIntosh concentrate was the best product tried. W. C. Cole

**533. A new flavor gets nationwide promotion.** Anonymous. *Ice Cream Trade J.*, 46, 4: 32, 92. Apr., 1950.

Chocolate almond ice cream has been introduced by the Borden Co. The almonds are chocolate coated and then injected into vanilla ice cream in the same manner as cherries or other fruits. Nationwide promotion has been placed behind the new flavor with full page advertisements appearing in several of the leading magazines. W. H. Martin

**534. Bulk ice cream in the profit picture.** W. D. DOBSON, Carnation Co., Los Angeles, Cal. *Ice Cream Trade J.*, 46, 4: 38-39, 84. Apr., 1950.

On the west coast there has been an increase in the sale of packaged ice cream, resulting in a decline in bulk sales as a percentage of total sales. Dealers have not pushed hand-packed ice cream.

The sale of soft ice cream also has cut into the sales of bulk ice cream. To cope with this situation, Carnation Co. has been holding dealer meetings for the purpose of teaching them to dip bulk ice cream and to make attractive fountain items. Dealers have been shown that a gross margin of 33 1/3% will result in increased sales and a greater net profit than resulted when a 43% gross was taken. Other dealer helps in the form of point of sale advertising, proper location of display cabinets and properly trained personnel should be provided as a means of increasing bulk sales. W. H. Martin

**535. Ice cream production is down three per cent from 1948.** Anonymous. *Ice Cream Field*, 55, 3: 66, 67. Mar., 1950.

The Bureau of Agricultural Economics of the U.S.D.A. estimates that the 1949 ice cream production was 553,705,000 gal. for the U. S. This amounted to a 3% reduction as compared to 1948. The largest percentage decrease occurred in New Jersey, whereas the southern states, as a group, showed the greatest decrease. Washington State showed a gain of 8% over 1948, which was the greatest increase shown by any 1 state. Sherbet production showed a 17% increase over 1948. Tabulated gallonages are given for the U. S. by months for 1949 and for states for 1948 and 1949. W. C. Cole

**536. What will the profits picture be in 1950?** L. C. ANDERSEN, General Ice Cream Corp. *Ice Cream Trade J.*, 46, 3: 28-29, 103. Mar., 1950.

Profits will be satisfactory in 1950 if ice cream manufacturers will refrain from giving unnecessary service and not offer items on which a profit cannot be made. Costs are likely to be up in 1950 because of increases in labor costs, increases in taxes and higher replacement costs, coupled with the possibility of reduced volume of sales. Costs on each item offered should be determined for the purpose of deciding whether or not the item should be sold. In figuring the cost of an item, material cost, manufacturing expense and truck and cabinet cost on basis of space occupied by the particular item should be considered. Greater operating efficiency and sales efforts may help to reduce costs and improve the profit picture. W. H. Martin

**537. Soda fountain operation. III. Menu.** A. C. DRAPER, Rexall Drug Co. *Ice Cream Field*, 54, 6: 40, 42, 44, 46, 48, 50, 51. Dec. 1949.

The author stresses the importance of planning the menu for a fountain before the fountain is planned. Location is important in deciding upon

the menu, but some items in the menu usually will increase the sale of others. If a fountain in a drug store sells ice cream items only, it will do an average of 5-10% of the store's business. Adding sandwiches may increase this to 12-15% and adding hot food can increase it to 20-30%. Adding hot food or any other service at a fountain necessitates planning for the problems that accompany such additions. In tabular form the author recommends the proportions of various items to use in various sized fountains. A discussion of costs, expenses and profits is given, and data reported in tables and charts serve as guides in determining these values. Examples in making such calculations are included. W. C. Cole

**538. Soda fountain operation. XII. Approach to layout.** A. C. DRAPER, Rexall Drug Co. Ice Cream Field, 55, 3: 22, 24, 53-55. Mar., 1950.

This is the concluding article in the series. The author outlines the considerations in deciding upon placement of fountain in store, type and shape of equipment and dimensions of equipment used. Drawings of the most common types of layouts are shown and the advantages and disadvantages of each type discussed. W. C. Cole

**539. Impulse buying of ice cream.** V. M. RABUFFO. Ice Cream Trade J., 46, 5: 28-29, 92. May, 1950.

A study of consumer buying habits in super markets in 7 cities indicated that 59.1% of ice cream purchases were completely unplanned. This fact shows the need for major emphasis on the point of sale suggestion for buying ice cream. The industry should concentrate on the points that will help influence the sale of ice cream when the customer enters the store. Some of the tools and devices which may be used include point-of-sale-posters, a lighted super structure over the ice cream cabinet, the location of the cabinet in a strategic place, accessibility of packages, attractive package design and insulated bags to protect the ice cream while in transit to the home.

W. H. Martin

**540. Selling ice cream through small town grocery store.** P. B. PERSON, Knerr Dairy Co., Fargo, N. D. Ice Cream Trade J., 46, 4: 44-45, 100. Apr., 1950.

The Knerr Dairy of Fargo, N. D., has been successful in building up its volume of ice cream sales through the use of newspaper and radio advertising to help its many small dealers in rural towns to sell more ice cream. Spot radio announcements and co-sponsored athletic events on the radio and advertisements in small town news-

papers and at the movie houses are some of the things which have been used as sales builders.

W. H. Martin

Also see abs. no. 511.

## MILK AND CREAM

### P. H. TRACY, SECTION EDITOR

**541. Will fat-free milk ruin your market?** W. L. FOUST, Warren, O. Milk Dealer, 39, 6: 138-141. Mar., 1950.

The sale of fat-free milk will not ruin regular milk sales unless it is sold as a cheap product; best results are achieved when the price is not more than 1¢ under the price of regular milk. A survey showed that 32% was sold as a baby food on doctors' orders, 18% was being used by women during pregnancy because of its high calcium content and low fat, 36% was being used by persons on reducing diets and the remaining 14% was being used by the lower income groups because of the slight economic advantage. Members of the medical profession point out that about 20% of the people today should be using this type product since it is protein they need most, not fat; therefore, it is reasonable to assume that about 20% of bottled milk sales could be sold in this manner. If the value of skim milk and cream is utilized by selling more low-fat milk at a reasonable price, then butter can be sold at a comparative price of oleo and still make money. There is a definite place in the market for this product and if the price is kept up so that a profit is made and not sold as an economy package, new markets can be captured. There has been a greater consumer acceptance with a low-fat, high-solids milk than with a purely fat-free milk.

C. J. Babcock

**542. A proposed new method of evaluating milk.** W. W. FASSETT, Sacramento, Cal. Milk Dealer, 39, 6: 45, 90-96. Mar., 1950.

The Jacobson theory that for every increase of 0.1% in fat test an increase of 0.04% SNF occurs is cited. This theory is based on averages of 100,000 tests. According to this theory, 3% milk has an SNF content of 8.27%. Therefore, for each pound of fat in 3% milk there would be 2.75 lb. of SNF. Since each increase of 0.1% in fat means an increase of 0.04% SNF, then 5% milk would contain 9.07% SNF. The advantages of evaluating milk by the SNF-fat combination are as follows: (a) All types of milk would sell on an equal basis as far as both fat and SNF are concerned. (b) It would equalize the purchasing power of plants in localities which are receiving milk from milksheds on which varying types of milk are produced. (c) It would make it pos-

sible to pay full value for all types of milk instead of underpaying on 1 type to make up for over payment on others. The following objections have been raised: (a) Some milk is claimed to have a higher vitamin content than others. (b) Some milk has a color intensity which is desirable for trade and for which some plants are willing to pay a premium. (c) Some milk is claimed to be more digestible than others. C. J. Babcock

**543. Container with pouring throat and connecting dispensing opening.** G. C. RIED and S. S. JACOBS. (Assignors to American Can Co.) U. S. Patent 2,499,416. 5 claims. March 7, 1950. Official Gaz. U. S. Pat. Office, **632**, 1: 113. 1950.

An improved pouring opening for paper containers for milk and other liquids, consisting of a cover-all flap to protect the pouring lip and the corner of the container molded in a rounded manner to facilitate pouring is described.

R. Whitaker

**544. Single closure for bottles.** C. H. KREBS. (Assignor to Standard Cap and Seal Corp.) U. S. Patent 2,501,849. 7 claims. March 28, 1950. Official Gaz. U. S. Pat. Office, **632**, 4: 1093. 1950.

This cap for milk bottles comprising metal foil, laminated on the outside to paper and on the inside to machine glazed paper, extends over and protects the entire pouring lip. By having the glazed surface of the inner layer next to the foil and the rough side next to the bottle, any difference in pressure between the exterior and interior of the bottle is equalized, but the product does not leak, as this layer is gas permeable and liquid impermeable.

R. Whitaker

**545. Supplementing fluid cream with frozen cream.** H. V. ATHERTON, Univ. of Vt., Burlington. Milk Dealer, **39**, 6: 157-158. Mar., 1950.

Preliminary results in the use of frozen cream to produce a 40% cream which will whip and which can be standardized down to 18% to produce a satisfactory coffee cream indicate that cream should be frozen and stored with a 40% fat content rather than as 50% cream, as is practiced for the ice cream industry. Best results are obtained by combining fresh cream and frozen cream on a 50-50 basis, heating the mixture to 140° F. or higher and then homogenizing at 100 lb./in.<sup>2</sup>, single stage. The resulting mixture appears to be entirely satisfactory for commercial usage.

C. J. Babcock

**546. Undersøgelser over Piskningen af mager Fløde.** (Research on the whipping of cream having a low fat content.) A. J. OVERBY, Royal

Vet. & Agr. Dairy Laboratory. Mælkeritidende, **59**, 43, 44, 45. 1946.

In Nov., 1940, a Danish regulation provided that cream to be sold retail must not contain more than 20% fat. On Feb. 10, 1943, the rule was changed to 15% in cream sold retail. Consumers were not able to whip 15% cream satisfactorily. It had been made unlawful, in 1925, to add any whipping aid to cream.

A good whipped cream must have a fine aroma, flavor and appearance. The foam must have a certain firmness and be of a definite volume, while no wheying off should occur after standing for a time.

The volume increases with an increase in the fat percentage until an optimum fat percentage is reached for stabilizing the foam, which is formed only from the liquid phase of the cream; therefore, less foam will be formed when the fat percentage is high.

Cream contains more foam substance (Skumstof), which is not composed of casein and albumin, but is the "membraneslime" that surrounds the fat globules. During whipping, many small foam lamellae, the walls of which must be strong, serve to hold the foam firm. The fat in satisfactory whipped cream should be present in small aggregations; large fat aggregates are undesirable as they cannot find a place on the lamella walls. The Danish experiments confirmed Hening's and Dahlberg's findings that it was possible to increase the viscosity and improve the whipping property of low-testing cream by reheating it. Cream of 15% fat was pasteurized and cooled to 2° C. (35.6° F.) and held at this temperature for 3 hr. The cream was slowly heated to 28-29° C., held at this temperature for 0.5 hr, cooled to 2° C. (35.6° F.) and left at this temperature until the following day.

Dalberg's and Hening's findings that superior whipping property and greater viscosity of cream resulted when the milk was separated at 5° C., as compared with separating at 50° C. were confirmed. Cream obtained from milk that had been frozen had a poor whipping property.

For economical and technical reasons it might prove of benefit to reheat cream having a high fat content, cool it and then standardize with cold skim milk to the desired fat content. Reheating gives the best results when cream having a high fat percentage is used. When cream of a higher fat content for whipping purposes comes into general use again, the method of reheating the cream for increasing its whipping properties would have definite significance. By use of the heat treatment method, marketing 20% cream that has as good a whipping property as 30% cream is possible.

Eleven tables and 2 illustrations are given in the article. There are 23 references. G. H. Wilster

**547. 3-day-a-week retail delivery.** W. HOLM, Sec., Columbus Milk Distributors Assn., Columbus, O. *Milk Dealer*, 39, 7: 146-151. Apr., 1950.

Going from every-other-day delivery to 3-d.-a-week delivery eliminates Sunday delivery. The advantages of eliminating Sunday delivery are lower labor costs, fewer relief problems, employees like it, 52 less operating days for the plant and consumers like it. C. J. Babcock

Also see abs. no. 475, 497, 506.

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**548. The excretion of steroid hormones concerned with controlling reproductive processes in animals.** H. H. COLE, Univ. of Cal, Davis. *Am. J. Vet. Research*, 11, 39: 161-165. Apr., 1950.

This paper is a review citing 80 references concerning the excretion of estrogens, androgens and progesterone. A critical discussion is presented of the observations concerning estrogen excretion by the cow in urine and feces and androgen excretion in cattle feces. E. W. Swanson

Also see abs. no. 498, 499.

## SANITATION AND CLEANING

K. G. WECKEL, SECTION EDITOR

**549. Modern materials and methods for dairy sanitation.** L. L. LITTLE, E. F. Drew and Co., Inc., Boonton, N. J. *Milk Plant Monthly*, 39, 4: 42-44, 46. Apr., 1950.

Although physical force is largely responsible for removal of soil from dairy equipment, scrub-

bing can be reduced greatly by compounding cleaners for specific jobs. Protein removal by chemical action of alkali cleaners can be facilitated by use of wetting agents. Grease films which necessitate saponification are removed more readily after emulsification. Mineral deposits usually are removed by acid cleaners; these deposits can be prevented by alkali cleaners in combination with wetting agents. Although mildly alkaline all-purpose cleaners are used for cleaning equipment in dairy plants, different methods for applying these compounds must be used for the varied pieces of equipment. These methods are vat solution, solution pail, dry powder, solution spray and circulation. J. A. Meiser, Jr.

**550. Het verband tussen stalinspectie en melk-kwaliteit. (The relationship between the judging of farm conditions and the quality of the milk.)** (English summary.) H. HEERES, State Dairy Organization, The Hague, Holland. *Neth. Milk and Dairy J.*, 4, 1: 10-20. Jan.-Mar., 1950.

The relationship was determined from studies of 10,200 farms producing market milk in the west part of Holland in the milk year 1948-1949. The result of judging of farm conditions was expressed as 8 for the best and 1 for the poorest. The quality of the milk was determined by methylene blue test and sediment test and given 3 for the best and 1 for the poorest quality. Yearly figures from 52 determinations varied between  $52 \times 3$  and  $52 \times 1$ . A correlation coefficient of  $-0.4 \pm 0.009$  was found, using the individual figures. In calculating the average quality figures for the 8 judging classes and employing these 8 figures, the correlation coefficient increased to  $-0.995$ .

On the average, a close relationship exists; however, in single cases other influences cause complications, making it impossible to calculate one factor from the other for individual cases with a proper degree of certainty. Under these circumstances, it is advisable to give attention to both factors. A. F. Tamsma

Also see abs. no. 483.



# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

### BOOK REVIEWS

**551. Farm Structures.** H. J. BARRE AND L. L. SAMMET. John Wiley & Sons, Inc., New York, N. Y. 650 pp. \$7.00. 1950.

Although written primarily for professional agricultural engineering students with a background in mechanics and strength of materials, this book contains considerable amounts of material of interest to those with less specialized background. Properties of structural materials, heating and heat transfer, moisture condensation and humidity, calculations of structures needed for different purposes and principles of satisfactory arrangement are covered. Factors concerned in the design and construction of many special types of buildings, including dairy barns and milk houses, are presented in a series of special chapters. In a number of cases, factors to be considered in the design of small milk plants are mentioned briefly. Material on space and structure demands for various operations in dairy barns and other buildings is summarized in readily understandable form. This should be an extremely valuable reference book for those having any interest in farm buildings.

F. E. Nelson

**552. Advances in Enzymology**, vol. 10. F. E. NORD, editor. Interscience Publishers, Inc., New York, N. Y. 533 pp. \$7.50. 1950.

The reviews included are: Blood clotting and related processes, by T. Astrup; Tryptophanase-tryptophan reaction, by F. C. Happold; Phosphatase alkaline, by J. Roche and Nguyen-van Thoai; Synthesis of disaccharides with bacterial enzymes, by W. Z. Hassid and M. Duodroff; Some aspects of streptomycin and other *Streptomyces* antibiotics, by N. G. Brink and K. Folkers; Probleme des Citronensäurecyklus, by Martins and F. Lynen; Die Phytochemie des Schwefels, by T. Bersin; Chemical changes in the harvested tobacco leaf: Part II. Chemical and enzymic conversions during fermentation and aging, by W. G.

Frankenburg; and Assimilation of hydrocarbons, by C. E. Zobell.

Adequate author and subject indices for this volume are included. The individual reviews are well-documented and appear to measure up to the standards set by the earlier publications in the series.

F. E. Nelson

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**553. Prophylaxie contre la tuberculose bovine, production laitiere et B C G.** (Prophylaxis against bovine tuberculosis, milk production and "B.C.G.") G. THIEULFEN. Lait, 30, 293-294: 141-147. Mar.-Apr., 1950.

The problem of bovine tuberculosis in France and statutes bearing on the matter are reviewed. It is proposed that the only valid method of dealing with the problem is through the use of B.C.G. vaccine which procedure is discussed. Effectiveness of this method would depend upon changes in sanitary regulations and certain other laws.

S. Patton

### BUTTER

O. F. HUNZIKER, SECTION EDITOR

**554. Method of manufacturing butter.** H. A. TOULMIN, JR. (Assignor to Commonwealth Engineering Co.). U. S. Patent 2,505,654. 12 claims. April 25, 1950. Official Gaz. U. S. Pat. Office, 633, 4: 1270. 1950.

Cream first is concentrated by removing water after it is frozen. The cream then is thawed and finally churned to form butter.

R. Whitaker

Also see abs. no. 581, 587.

### CHEESE

A. C. DAHLBERG, SECTION EDITOR

**555. Sur la maturation des fromages.** (On the

ripening of cheeses). M. BEAN. *Lait*, 30, 293-294: 122-141. Mar.-April, 1950.

Polarization, titration and precipitation methods for estimating the maturity of cheeses and the extent and type of proteolysis taking place during ripening were studied and evaluated. It is indicated that the various methods leave something to be desired with respect to measuring the quantity and types of amino acids present at various stages of ripening and in various types of cheese.

S. Patton

556. **Manufacture of cheese.** G. W. McDONALD and E. C. SCOTT (assignors to Swift and Co.). U. S. Patent 2,507,480. 8 claims. May 9, 1950. Official Gaz. U. S. Pat. Office, 634, 2: 642. 1950.

The lactalbumin in whey is precipitated in large particles, homogenized and added to milk in amounts ranging from 1-50 parts of the casein. The modified milk then is curdled by an acid which produces a curd in which the added lactalbumin is uniformly dispersed. Cheese made from this curd has improved nutritional value and moisture-holding properties.

R. Whitaker

557. **Making a quality cottage cheese.** N. C. ANGEVINE, Meyer-Blanke Co., St. Louis, Mo. *Milk Dealer*, 39, 8: 51-52, 102-107. May, 1950.

Sales of cottage cheese have increased tremendously in the past several years, partly because of its current value as a replacement for other animal proteins in the human diet and partly because the consuming public is beginning to realize its real importance as a food. Also, a large portion of cottage cheese manufacturers are treating it as a major product, much the same as ice cream, butter and fluid milk. The quality definitely is being improved.

Based upon the 1930 census, cottage cheese consumption in 1925 was less than 0.5 lb./person. In 1935, it was 0.9 lb. Based on the 1940 census, the 1940 per capita consumption was 1.3 lb., the 1943 was 1.6 lb., and the 1947 estimated at 3.21 lb. The 1948 per capita consumption, based on probable 1950 census, is estimated at 3.36 lb. Methods of manufacturing cottage cheese are discussed.

C. J. Babcock

558. **Process for the manufacture of cream cheese.** R. P. CHENIER. U. S. Patent 2,508,663. 1 claim. May 23, 1950. Official Gaz. U. S. Pat. Office, 634, 4: 1166. 1950.

Cream cheese containing about 75% fat is made by pasteurizing cream-enriched milk with infrared rays, cooling and again treating with infrared rays, and holding for about 3 d. at 40° F. A rennet curd made from infrared pasteurized

milk is washed, drained and mixed with the aged cream and a stabilizer, such as gum tragacanth, gelose and pectin. The product is beaten to incorporate air in well dispersed small bubbles.

R. Whitaker

559. **Het Schreuder-Kaasvat.** (The Schreuder cheese vat.) (English summary.) Dutch Association of Co-operative Dairy Factories, The Hague, Holland. 18 pages. Feb., 1950.

A new type of cheese vat for Edam cheese, described in the Netherlands, patent no. 45643, was tried for usefulness and efficiency. It is constructed in such a way that the cheese, when it comes out of the brine, has flattened sides. This facilitates placing and turning of the cheese on the shelves and produces a much better shape of cheese with considerably less labor. There was some trouble with the cloth sticking to the cheese. Some factories experienced no particular difficulty in this respect. It may therefore, be possible for each factory to evolve its own special method to prevent sticking of the cloth. In this case, the use of the new type of cheese vat may be strongly recommended.

A. F. Tamsma

560. **Cheese making method.** R. MIOLLIS. U. S. Patent 2,505,984. 6 claims. May 2, 1950. Official Gaz. U. S. Pat. Office, 634, 1: 160. 1950.

Whey is drained from curd in 3 steps, some being removed in the vat in which the curd is formed, some as the curd is transferred to a 2nd vat and some while further drainage is accomplished in the 2nd vat. The curd finally is transferred to a 3rd vat for matting.

R. Whitaker

561. **Cheese merchandising and public relations.** T. B. COOPER. *Can. Dairy Ice Cream J.*, 29, 4: 86-89, 96. Apr., 1950.

The only market for Canadian cheese is Great Britain. There has been very little attempt to encourage greater home consumption. The Canadian consumption of cheese is approximately 4.2 lb./person/year. A great number of Canadians never buy any cheese in any quantity. To increase the consumption of cheese, a National Cheese Week was sponsored by the National Dairy Council of Canada. There is a need for consumer education on cheese and better merchandising methods.

H. Pynson

562. **Cheese merchandising.** O. R. IRVINE and W. H. SPROULE. *Can. Dairy Ice Cream J.*, 29, 5: 29-32. May, 1950.

Sales of process cheese were stimulated at the expense of natural cheese because of better packaging, greater uniformity, lack of waste, etc. During and since the war, a number of packag-

ing materials have been developed for packaging natural cheese. These comprise the transparent or semi-transparent films possessing heat-sealing properties and capable of being printed in attractive colors. A good package should: (a) protect the product, (b) identify the product, (c) have low cost, (d) be adaptable to machine application and (e) be light in weight. The Good-year Tire and Rubber Co. has developed the pliofilm pressure-pack method where 20- or 40-lb. square hoops are used and pliofilm and glassine paper wrapped around the cheese. When the cheese has been aged sufficiently, they are unwrapped, cut in 0.5-, 1-, 2-, or 5-lb. prints and double-wrapped in pliofilm. Wedge-shaped prints also have been developed by this method. Another method (Milprint, Inc.) cuts cheddars in wedges, the rinds are not removed. Each piece is weighed and marked separately. Kraft has developed a wax-coated cellophane. Special waxes also have been developed for retailing cheese. H. Pyenson

Also see abs. no. 566, 573.

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**563. Etude, au microscope electronique de la structure du lait en poudre "Spray."** (Electron microscope study of the structure of spray dried milk powder.) A. C. VILLANOVA and O. BAILARIN. *Lait*, 30, 293-294: 114-122. Mar.-Apr., 1950.

By means of an electron microscope, photomicrographs (published with the paper) of spray dried milk powder particles were obtained. Original enlargements of the particles by the microscope were on the order of 3,500-12,500 diameters. Further magnification, up to 50,000 diameters, was attained by photographic enlargement.

The results indicate that the milk components are distributed evenly throughout the powder particle. The fat is very highly divided and certain of the salts are crystallized. Lactose did not appear to be crystallized. S. Patton

**564. What's ahead of the concentrated milk industry in Canada?** D. B. GOODWILLIE. *Can. Dairy Ice Cream J.*, 29, 4: 52-56. Apr., 1950.

Consumption of concentrated milk products has increased materially the last 10 yr. and indications point to this trend continuing. Canada imports little concentrated milk. It exported in 1949 approximately 79 million lb., having a value of \$12.9 million. Evaporated milk buyers are not anticipating their requirements very far in advance, possibly expecting lower prices. The

sweetened condensed milk peak was reached in 1948 and production has dropped sharply since. Dry whole milk production, consumption and export have gone down considerably. Dry skim production last year was practically the same as in 1948. The amount of spray process produced has increased and the amount of roller process has decreased. Domestic consumption last year was one of the highest on record.

H. Pyenson

**565. The freezing point of reconstituted non-fat and dry milk solids.** J. G. FEATRO and A. V. MOORE, Texas Agr. Expt. Sta., College Station. *J. Milk & Food Technol.*, 13: 167-169. May-June, 1950.

Drying of skimmilk solids tends to decrease the solubility of certain milk constituents and thus raise the freezing point of the reconstituted product. When fresh normal whole milk is mixed equally with reconstituted non-fat dry milk solids, the freezing point of the blend will be comparable to whole milk, providing the reconstituted non-fat dry milk solids product is 9.3% solids. The freezing point does not detect a skimmilk made by reconstituting a non-fat dry milk solids of 9.3% solids. There appears to be no difference between the freezing point of freshly reconstituted non-fat dry milk and milk held at 45° F. for 48 hr. H. H. Weiser

**566. Use of nonfat dry milk solids in the making of cottage cheese and cultured buttermilk.** J. C. STILES, Golden State Co. *Milk Dealer*, 39, 8: 132-138. May, 1950.

A high per capita consumption of cottage cheese and buttermilk will result only when the consumer has a quality product 12 mo. a year. Use of low-heat nonfat dry milk solids offers the cottage cheese manufacturer a dependable source of solids to place production of a quality product on a year-round basis. The dependable source of uniform high-quality solids also will help to make buttermilk a profitable, year-round specialty. Directions are given for the use of low-heat dry milk solids in the preparation of cottage cheese and buttermilk. C. J. Babcock

**567. Reconstituting milk.** A. O. DAHLBERG. *Can. Dairy Ice Cream J.*, 29, 5: 36-37, 42. May, 1950.

For beverage use there has developed a market for reconstituted milk made from high quality, dry milk fat and low-heat, nonfat dry milk solids. This method has been used in Japan, South Pacific, Alaska and the Aleutians. Five milk reconstituting plants are now operating in Japan and 1 each on Okinawa and Guam. The U. S.



Army has certain specifications that must be met for the dry milk fat and nonfat dry milk solids. Reconstitution is accomplished with equipment ordinarily found in a creamery. The use of high quality raw materials can not be over-emphasized. The dry butterfat and the nonfat dry milk solids probably will be used in ice cream, also.

H. Pyenson

**568. Der Eiweissgehalt von Gärungsmolkgetränken. (The protein content of fermented whey beverages.).** (English summary.) K. KUMETAT. *Die Milchwissenschaft*, 4, 2: 53, 55 Feb., 1949.

Milone (a fermented whey beverage) and beer were analyzed for total N, tannic acid precipitation fraction of N (Lundin method) and formol titration value of N. Milone had an average total N value of 25 mg.%, whereas light beer had an average value of 64 mg.% and dark beer of 86 mg.%. In milone, 48% of the total N (12 mg.%) was precipitated with tannic acid as against 7.4% (5 mg.%) in light beer and 4.6% (4.1 mg.%) in dark beer. These values show that there were fewer protein degradation products in milone than in either type of beer. The average formol titration value for milone was 25% of the total N value (6.4 mg.%) and for light beer 31% (22 mg.%).

In the method of Lundin, 200 ml. milone were pipetted into a 25-ml. vol. flask. To this were added 6 ml. of 50%  $H_2SO_4$  and 12 ml. of 16% tannic acid, the mixture adjusted to 20° C., made to volume with water and filtered. The N-determination was made by the Kjeldahl method, using the condensate from 100 ml. of original filtrate. I. Peters

**569. Method of producing a lacteal beverage.** E. C. SCOTT (assignor to Swift and Co.). U. S. Patent 2,507,482. 2 claims. May 9, 1950. Official Gaz. U. S. Pat. Office, 634, 2: 643. 1950.

A milk shake having a texture and body similar to a milk shake prepared from ice cream is made from a dry mix containing 15-35% butter fat, 20-40% milk solids-not-fat and 20-40% sugar. The powder first is made into a paste, using 25-75 parts water to 100 parts powder. Flavoring then is added and the finished drink prepared by briskly agitating 75-200 parts of chopped ice with 100 parts of dry powder. R. Whitaker

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**570. Heat resistant bacteria in raw milk. Part I. Comparison of thermoduric colony counts on yeastrel milk agar incubated for 2 days at 37° C.**

and 4 days at 30° C. S. B. THOMAS, DOROTHY ELLISON, D. G. GRIFFITHS, E. JENKINS and K. J. MORGAN. *J. Soc. Dairy Technol.*, 3, 3: 187-190. Apr., 1950.

Milk samples were pasteurized in the laboratory at 63.5° C. for 35 min. before plating. Over 80% of the samples had higher counts at 30° C. than at 37° C. Samples with low counts at 37° C. showed the greatest difference between the 2 temperatures, while samples with high counts at 37° C. showed relatively slight difference between 30 and 37° C. It was assumed that the larger differences between the 2 counts were due to the presence of microbacteria and certain micrococci incapable of growing at 37° C., while small differences were due to the presence of a relatively high proportion of thermoduric streptococci that could grow at both temperatures.

The authors recommend an incubation temperature of 30° C. for thermoduric plate counts of milk, utensil rinses and swabs instead of or in addition to the 37° C. ordinarily used.

E. M. Foster

**571. Heat resistant bacteria in raw milk. Part II. Grading farm milk supplies by keeping quality and thermoduric colony count.** S. B. THOMAS, DOROTHY ELLISON, D. G. GRIFFITHS, M. HUMPHREYS, W. L. R. VAUGHAN, G. GEORGE and E. P. DAVIES. *J. Soc. Dairy Technol.*, 3, 3: 190-195. Apr., 1950.

Over 3600 samples of milk were examined for numbers of thermoduric bacteria and for keeping quality at 20° C. Thermoduric counts were obtained from yeastrel agar plates incubated at 30° C. Keeping quality was expressed as time required to clot on boiling. The results showed a general association between the 2 tests in that a high proportion of samples of good keeping quality had low thermoduric counts and nearly half the samples of poor keeping quality had high thermoduric counts. A system of grading milk into 4 classes on the basis of these 2 tests is suggested. The difficulties in interpreting the results are recognized and suggestions are made to aid in their interpretation. E. M. Foster

**572. Types of organisms present in commercially pasteurized milk.** J. W. EGBELI and E. R. BIRD. *J. Soc. Dairy Technol.*, 3, 3: 171-177. Apr., 1950.

From 11 dairies in the west of England, 21 samples of milk were taken directly from the pasteurizers and another 21 samples representing the same batches were taken after bottling. Each sample was plated on yeastrel milk agar and plates incubated at 22, 30 and 37° C. Colonies were

picked at random from the plates and the organisms identified.

Average counts at 22 and 30° C. were 4-5 times higher than were those at 37° C. Of the isolants from the 37° C. plates, 48% were streptococci, 28% were micrococci and 16% were aerobic spore-forming rods. However, the cultures from the 30° C. plates were distributed thus: 17% microbacteria, 20% micrococci, 16% streptococci and 12% aerobic spore-forming rods. The proportion of microbacteria in the 22° C. plates was even higher (65%) with micrococci (17%) and streptococci (10%) accounting for most of the remainder. Practically all of the microbacteria were *Microbacterium lacticum*, while *Streptococcus thermophilus* accounted for all but 1 of the streptococci identified. The micrococci and aerobic spore-forming rods were distributed among several groups and species.

*Streptococcus lactis* and coliform organisms were not found in 1-ml. quantities of the samples immediately after pasteurization but were found in the milk after storage at 18° C. This was not believed to indicate post-pasteurization contamination but, rather, that these organisms survived the commercial pasteurization in very small numbers and then grew during storage.

The authors suggest that the reason for lower counts on pasteurized milk plates incubated at 37° C than at 30° C is due to the failure of microbacteria and certain micrococci to grow at the higher temperatures. E. M. Foster

**573. Cultures and starters for cheesemaking.** M. W. HALES. Can. Dairy Ice Cream J., 29, 5: 50-60. May, 1950.

This article covers (a) functions of bacteria in lactic cultures, (b) selection of milk, (c) heating milk, (d) inoculating milk, (e) incubation of cultures and starters, (f) ripening, (g) influence of cooking temperatures on acid development in cheesemaking and (h) bacteriophage.

H. Pyenson

**574. Ein Pilznährboden als vollwertiger Ersatz für Fleisch- und Pepton-Nährböden.** (A fungus growth medium as substitute for meat infusion or peptone media.) (English summary.) W. KUNDRAT. Die Milchwissenschaft, 4, 2: 55-56. Feb., 1949.

A number of test organisms, including bacteria, yeasts and molds, grew equally as well or better on a mushroom infusion agar than on such commonly used media as meat infusion agar, nutrient agar or acidified potato dextrose agar. Equally good growth of test organisms was obtained by using the tops of (a) edible mushrooms only, (b)

both edible and non-edible and (c) a very bitter mushroom (*Boletus felleus* Bull.).

The mushroom infusion agar was prepared by boiling 500 g. of mushroom tops in 500 ml. of tap water, filtering and adjusting the filtrate with additional water to 1 l. Two per cent agar was added to the filtrate and the medium sterilized in the regular manner. When air-dried ground mushrooms were used, the equivalent of 350-400 g. of fresh mushrooms/l gave optimum microbial growth, thus resulting in a saving of from 20-30% of mushrooms. I. Peters

**575. Penicillin, as an adjunct to the preservation of quality of raw and pasteurized milk.** E. J. FORRY and J. V. BYRNE, Wallace Research Laboratories. J. Milk & Food Technol., 13, 170-174. May-June, 1950.

The effects of penicillin were studied on the bacterial counts of raw and pasteurized milks held at different temperatures under laboratory conditions. The results are not conclusive. However, the study shows that 3 units of penicillin/ml. can suppress the growth of certain species of bacteria in milk held at the most favorable temperature for bacterial growth. H. H. Weiser

**576. Orotic acid, a growth factor for *Lactobacillus bulgaricus*.** L. D. WRIGHT, J. W. HUFF, H. R. SKITGES, K. A. VALENTIK and D. K. BOSSHARDT, Sharp & Dohme, Inc., Glenolden, Pa. J. Am. Chem. Soc., 72, 5: 2312-2313. May, 1950.

Some strains of *Lactobacillus bulgaricus*, such as strain 09 of the Cornell collection, require another factor(s) besides L.B.F. Yeast extract and whey are good natural sources of the growth promoting substance. At a level of 10-100 $\gamma$ /tube, orotic acid (uracil-4-carboxylic acid) was found to replace the need for large amounts of natural material. The factor could not be replaced by uracil, uridine, uridylic acid, cytosine, cytidylic acid, uric acid, asparagine, aspartic acid, lactose, urea, alloxan, allantoin, thymine,  $\gamma$ -aminobutyric acid, 5-carboxyuracil, 4-methyluracil and 2-amino-4-methyl-6-hydroxypyrimidine. H. J. Peppler

**577. The iron requirement of rumen bacteria.** (Abs.) MARY L. MCNAUGHT and E. C. OWNE, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., 44, 3: xxiv. 1949.

Studies of rumen bacteria *in vitro* indicate that about 1.0 ppm. of iron is essential for their growth.

A. O. Call

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**578. The semi-micro estimation of lactose alone**

and in the presence of other sugars. F. H. MALPRESS and A. B. MORRISON. Queen's University, Belfast. *Biochem. J.*, **45**, 4: 455-459. 1949.

This method for estimation of small amounts of lactose is based on the color reaction between lactose and methylamine in alkaline solution. The developed color, following a strict time schedule, is measured in a colorimeter. The method is most sensitive in the range of 0.05-0.2%. Some discussion is given regarding interfering substances.

A. O. Call

**579. Qualitative scheme of analysis for the common sugars.** T. H. WHITEHEAD and W. C. BRADBURY, Univ. of Georgia, Athens. *Analyt. Chem.*, **22**, 5: 651-653. May, 1950.

Mixtures of sucrose, glucose, fructose, maltose and lactose in solid and liquid samples may be analyzed qualitatively without the use of special equipment or unusual techniques and reagents. Fructose is removed from the solid sample with 90% ethyl alcohol. Lactose, starch and dextrans next are removed from the residue with 50% alcohol and the presence of lactose is confirmed with the formation of its osazone. The filtrate, after removal of the lactose residue contains sucrose, glucose and maltose. Sucrose may be detected by its color reaction with cobalt nitrate, and glucose and maltose are separated by forming the osazones. Five mg. of sucrose and fructose or 200 mg. of the other sugars may be detected in a sample.

B. H. Webb

**580. Fat determinations in milk.** L. GERSHENFELD and B. UCKO, Dept. of Bacteriology, Philadelphia Coll. of Pharm., Pa. *J. Milk & Food Technol.*, **13**: 175-176. May-June, 1950.

The authors claim that the Schain method for determining fat content in raw, pasteurized plain and homogenized milks has many advantages over the Babcock test. The test involves the mixing of the sample with solution A (oil red O in isopropyl alcohol with a non-ionic detergent in ethyl alcohol) in a Babcock bottle. Reagent B (a standardized anionic detergent) is added without shaking and the mixture placed in a water bath at 180° F. for exactly 5 min. Water at 180° F. also is added to bring the liquid up to the graduated portion of the neck. The mixture is allowed to stand at room temperature for 20 min. before reading.

In order to compare the fat readings with either the Babcock or Roesse-Gottlieb methods, it is necessary to standardize solution A in the Schain procedure for each type of milk. The Schain method is not applicable to buttermilk, creams, ice cream or other milk products.

H. H. Weiser

**581. Rancidity in Indian butterfats (Ghee).** K. T. ACHAYA, Univ. of Liverpool. *Biochem. J.*, **44**, 5: 561-567. 1949.

Indian butterfat (Ghee) from 3 sources, (a) buffaloes on normal ration, (b) buffaloes heavily fed cottonseed under arid conditions and (c) normal cow butterfat, stored under usual unrefrigerated conditions for a period of 3-4 yr., were used in the study. The common fat constant determinations were made on pooled rancid samples from the 3 sources. Increases in acidity, Polenske, Reichert and saponification numbers, and decreases in iodine values occurred in all cases. An oxidative mechanism producing free fatty acids is suggested in the case of these butter oils as opposed to mainly lipolytic changes in butter on becoming rancid.

Results of the free fatty acids determinations are given in a table. The absence of oleic acid in quantities as large as expected and the presence of unsaturated, nonvolatile residues suggest that polymers were formed during the prolonged storage.

A. O. Call

**582. An immune globulin fraction from bovine precolostrum.** E. I. McDUGALL, Univ. of Cambridge. *Biochem. J.*, **44**, 5: 531-541. 1949.

Bovine precolostrum is described as "a viscous honey-like substance obtained from the udders of pregnant heifers at half term." Schematic details of fractionation procedures by salting out are given. The study deals mostly with that fraction salted out by 33% saturation ( $\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> and referred to as an immune globulin. Electrophoretic measurements showed the fraction to contain only 1 component, but solubility tests indicated more than 1 component. A nitrogen content of 15.15% and an apparent molecular weight of 300,000 are given. Although not pure, the fraction represents the main component of precolostrum exhibiting immune properties. The similarity and interrelation of this globulin to those of colostrum and milk are pointed out.

A. O. Call

**583. Protein reaction product preparation.** J. P. DANEHY (Assignor to Harris-Seybold Co.). U. S. Patent 2,500,453. 13 claims. March 14, 1950. *Official Gaz. U. S. Pat. Office*, **632**, 1: 492. 1950.

To facilitate the production of casein plastics and other products, the casein is dispersed, not in water as is the usual medium, but in compatible organic agents such as glycols, glycerol, acid amides and certain phenols. Aldehyde precipitation then is accomplished in the temperature range of 110-140° C.

R. Whitaker

**584. pH in the dairy industry.** J. G. DAVIS. *Food*, 19, 222: 84. Mar., 1950.

This is a second review paper on pH relationships and test procedures of platform testing of milk, and effect of pH on the physical structure of milk. K. G. Weckel

Also see abs. no. 555, 565, 619.

## DAIRY ENGINEERING

A. W. FARRELL, SECTION EDITOR

**585. Developments in small tube heat exchangers.** F. O. HERREID. *Can. Dairy Ice Cream J.*, 29, 5: 43-44, 92. May, 1950.

The small-tube heat exchanger at the University of Illinois has a capacity of 212 gal./hr. at 2000 lb. pressure. The heating section has 143 ft. of stainless steel tubing, 0.25 in. diameter. The cooling section is of the same construction, and water is used as the cooling medium. The temperature in the heating section is controlled by a Fulscope. The velocity of the product through the entire unit is 23.1 ft./sec. It takes 12.34 sec. to heat and cool the product. The heat exchanger has been used in processing milk, cream and ice cream mix. Test organisms were used in this study. Cream heated to 240-300° F. was made commercially sterile; satisfactory phosphatase values were obtained at 190° F. and above. High velocities reduced the size of the fat globules in cream from 3.1-2.1  $\mu$  at a temperature of 300° F. The heat exchanger produced no changes in acidity and hydrogen ion concentration through a temperature range of 170-300° F. for 6.1 sec. at each temperature.

The viscosity of the cream was reduced by about 50% through the temperature range of 200-300° F., while through the range of 170-190° F. the reduction was about 40%. High temperatures did not affect the stability of cream in coffee, although it did have more coloring ability in coffee than raw cream. Cooked flavor became slightly evident at 210-220° F. and pronounced at 240-260° F. but was not objectionable even up to 300° F. The whipping time of the cream was not changed measurably by heating it to 300° F. When the curd tension was reduced by more than 20%, a slight cooked flavor was obtained; when it was reduced to zero at temperatures above 240° F., the flavor of the milk was definitely cooked. With milk a cooked flavor was much less than that found in evaporated milk. In ice cream the whipping time was prolonged, but this condition could be corrected by using an emulsifying agent, in addition to a stabilizer. The best temperatures at which to process ice cream mix in the heat exchanger were 240-

250° F. from a flavor standpoint. Heat-resistant vegetative forms in ice cream were destroyed at 170-180° F. Spore formers were destroyed at 240-260° F. Above 180° F. all phosphatase tests were negative. Due to high velocity, there is no laminar flow but a turbulent flow, which prevented cooking the product onto the heating surface. The heater exchanger (Mallorizer) is cleaned easily by circulating alkaline and acid solution through the tubes under pressure.

H. Pyenson

**586. Dumping device for milk cans.** H. M. KENDALL. U. S. Patent 2,509,393. 12 claims. May 30, 1950. Official Gaz. U. S. Pat. Office, 634, 5: 1466. 1950.

A tilting device, attached to the end of a conveyor, permits easy emptying of 10-gal. milk cans into a weigh vat or other vessel. R. Whitaker

**587. Self-washing cream separator.** W. H. HARTSTICK (Assignor to International Harvester Co.). U. S. Patent 2,504,261. 22 claims. April 18, 1950. Official Gaz. U. S. Pat. Office, 633, 3: 789. 1950.

A centrifugal type cream separator is described, consisting of the usual series of disks within a rotating bowl, so designed and equipped with a rotating tube extending upward through the supply tank that the bowl can be flushed with a cleaning fluid without disassembly.

R. Whitaker

**588. Milk and cream product emulsifier.** J. H. GARDNER (Assignor to Elizabeth Gardner). U. S. Patent 2,504,678. 13 claims. April 18, 1950. Official Gaz. U. S. Pat. Office, 633, 3: 896. 1950.

An homogenizer valve in which the milk or cream under high pressure is forced radially outward between perforated disks which are pressed together by a hand screw is described.

R. Whitaker

**589. Freezing machine.** A. J. TACCHIELLA (assignor to Steady Flow-Freezer Co.). U. S. Patent 2,508,435. 16 claims. May 23, 1950. Official Gaz. U. S. Pat. Office, 634, 4: 1107. 1950.

An ice cream freezer for delivering, intermittently, individual portions of ice cream is described. A foot-actuated mechanism, when depressed, opens the freezer gate valve at the front end of the freezer and also opens a valve at the back end admitting an amount of mix in proportion to the amount of ice cream withdrawn, thus maintaining the optimum amount of ice cream in the freezer at all times.

R. Whitaker

**590. Process for washing containers.** H. D. LATHROP and V. SCHWARZKOPF (assignors to Lathrop-Paulson Co.). U. S. Patent 2,509,003. 8 claims. May 23, 1950. Official Gaz. U. S. Pat. Office, 634, 4: 1252. 1950.

A milk can washer which employs a cleaning compound of the type which makes a foam with the milk solids adhering to the walls of the cans is described. The liquid and foam drain into a sump, from the bottom of which the liquid is reused. The foam overflows as some rinse water gains access to the sump. R. Whitaker

**591. Tools for saving water.** H. E. DEGLER, Marley Co., Kansas City, Kan. Power, 94, 5: 102-106. May, 1950.

Evaporative cooling is an effective method of conserving cooling water. Spray ponds are costly in space and are relatively inefficient. Many have been replaced by cooling towers and dry coolers.

Spray-filled natural-draft towers serve for cooling requirements of less than 30,000 Btu/min. The atmospheric deck tower is a modification which breaks and rebreaks the fall of the water. These towers should be broadside to prevailing winds and are inefficient in wind velocities under 3 mph.

Mechanical-draft towers fan-produce air movement and are more compact and efficient. Air passes counterflow or across the water streams. In mechanical draft towers, water is either spray-filled or cascaded over wood fillers. They may use combinations of both. Exhaust air is passed through a drift eliminator to remove entrained moisture.

Forced-draft towers are suitable for corrosive waters. The fan is mounted at near ground level, and the warmed air leaves at low velocity at the top of the tower. The warm air may find its way back to the fan inlet and cut performance of tower.

Induced-draft towers have the fan at the top and draw air horizontally across or upward through the filling. The water basin on this type is accessible for cleaning during operation.

Dry coolers may be used where fluid temperature starts above 140° F. or where water is scarce, expensive or badly polluted. The fluid is circulated through finned coils over which air passes.

Illustrations show the main features of these towers and tables present relative cost figures.

H. L. Mitten, Jr.

**592. Water treatment for cooling towers.** J. B. DAVIS, Allis-Chalmers Mfg. Co., Milwaukee, Wis. Heating, Piping & Air Cond., 22, 4: 89-93. Apr., 1950.

Advantages and disadvantages of once through,

closed and open cooling systems are discussed. Cooling system problems in probable order of importance are: (a) scale deposition or fouling, (b) corrosion of metal surfaces and (c) formation of organic growths. Scale preventive procedures are classed as: (a) softening, (b) alkalinity reduction, (c) surface active treatment, (d) deconcentration, (e) sterilization.

Selection of water treatment should be based on availability of water, number of times water can be concentrated without scale deposition and cost of treatment. Tables and diagrams presented give data which can aid in treatment selection. H. L. Mitten, Jr

**593. Rapid test for calcium hardness.** V. M. MARCY, Hall Laboratories, Inc., Hagan Bldg., Pittsburgh, Pa. Power, 94, 6: 92-93. June, 1950.

The method described requires no special techniques, may be used with waters containing as little as 0.5 ppm. Ca and is rapid and accurate. Reagents required are available commercially. They consist of a buffer which is a strong NaOH solution containing  $\text{Na}_2\text{S}$ , an indicator made by adding stabilized ammonium purpurate to distilled water and the titrating solution which contains a complex-forming salt of ethylene-diamine-tetra-acetic acid.

The procedure is as follows: (a) Measure 50 ml. of water sample into a white porcelain cup and add 4 drops of buffer solution to adjust pH to about 12. (b) Add 1-3 drops of indicator. This indicator is violet-blue at pH 12 in Ca-free water, red if Ca is present. (c) Titrate with a standard solution of ethylene-diamine-tetra-acetic acid salt until the violet-blue end point is obtained. (d) Compute results according to the strength of the titrating solution used.

H. L. Mitten, Jr.

**594. Shooting trouble in a refrigeration system.** G. Holman. Heating, Piping & Air Cond., 22, 4: 100-102. Apr., 1950.

A case history of an ice-building, sweet water system in a dairy is presented. The system had not been properly purged of air and pressurestats needed adjusting. H. L. Mitten, Jr.

**595. Are your motors overheating?** J. L. WATTS, Southampton, England. Power, 94, 5: 87-89. May, 1950.

When the supply voltage and motor load are constant, the motor temperature increases gradually and eventually reaches a constant level. Maximum temperature rises are held within allowable limits of motor insulation. When safe temperatures are exceeded, the life expectancy of the motor is reduced. Motors installed in excep-

tionally warm atmospheres should be given reduced loads to keep within safe temperature limits.

In general, the factors which cause motors to overheat are overloading, low voltage at motor terminals, worn bearings, failure of starting winding to "kick out," inadequate motor ventilation, high starting currents over a relatively long time, one line of a 3-phase circuit open, incorrect motor connections and electrical faults in the motor.

Each motor should be protected with an over-current device which will open the line contactor if an overload is sustained.

Mechanical defects which may cause overheating or overload are improper lubrication of bearings, excessive belt tension and shafts not aligned. A large percentage of motor breakdowns can be avoided by periodic inspection and early correction of faults. H. L. Mitten, Jr.

Also see abs. no. 551, 554, 559.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**596. A new approach to plant planning.** G. R. JOHNSON, Pace Associates, Chicago, Ill. *Milk Dealer*, 39, 8: 48, 74-75. May, 1950.

To obtain adequate and efficient milk and ice cream plant facilities, determine existing requirements and estimate the rate of expansion. A basic criterion in determining whether or not to build or remodel is that of flexibility to allow for changes in production methods. Tomorrow's processing techniques may vary significantly from today's, and equipment layout and floor areas may produce different requirements. New facilities will offer definite advantages, such as efficient operation, flexibility of production techniques, ease of maintenance, tax savings and publicity value. C. J. Babcock

**597. Pallet system of handling cases of milk.** Anonymous. *Milk Dealer*, 39, 8: 144-146. May, 1950.

The modern technique of fork-truck pallet handling of milk cases not only has saved time but has created space for storage and other essential uses, has greatly reduced the traffic congestion on the loading docks and has sharply reduced the incidence of breakage and loss of product and bottles at the Whiting Milk Co., Boston, Mass. C. J. Babcock

**598. Billing method cuts statement time.** Anonymous. *Milk Dealer*, 39, 8: 44-45. May, 1950.

The simplified system of monthly account statements used by the Pioneer Dairy, Great

Falls, Mont., is described. The advantages of the system are: (a) It gives a much nicer looking statement than does hand posting. (b) By using a posting machine and beginning to post around the 24th of the month, 1 bookkeeper can handle 1,800 accounts and do all the other plant book work. (c) By posting the total on the statement copy and not on the original, the customer receives dated statement with no evidence that it has been done at 2 times and (d) there has never been a time when statements were not out on time and without extra help. C. J. Babcock

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**599. The absorption of vitamin A.** EVA EDEN and K. C. SELLERS. Univ. of Cambridge *Biochem. J.*, 45, 5: xxxiii. 1949

Twenty cows and sheep of various ages were fed vitamin A alcohol and vitamin A ester at the rate of 5000 I.U./kg. body weight. The animals were slaughtered 4 hr. after dosing, and vitamin A alcohol and ester were determined on the intestinal contents and walls and in the lymph. Whether given in the form of ester or alcohol, the vitamin A was absorbed principally in the ester form. A. O. Call

**600. The absorption of vitamin A in ruminants and rats.** E. EDEN and K. C. SELLERS. Univ. of Cambridge *Biochem. J.*, 44, 3: 264-267. 1949.

Vitamin A in the form of halibut liver oil was fed at a level of 5000 I.U./kg. body wt. to 16 bullocks, 19 adult sheep and 60 rats. The blood plasma vitamin A was estimated before dosing. At intervals ranging from 2-24 hr. after dosing, the animals were sacrificed and the systemic and portal blood, as well as the lymph, or lymph glands, were tested for vitamin A. In ruminants and rats vitamin A is absorbed principally through the intestinal lymph, especially in the upper part of the intestine. Portal blood (from the intestines to the liver) failed to show a significant rise. A. O. Call

**601. Effect of the prepartal diet of the cow on the placental and mammary transfer of tocopherols to the calf.** D. B. PARRISH, G. H. WISE, C. E. LATSCHAR and J. S. HUGHES, Kansas Agr. Expt. Sta., Manhattan. *J. of Nutrition*, 40, 2: 193-202. Feb., 1950.

The tocopherol levels in the blood serum of calves from dams with and without vitamin supplements during the terminal stages of gestation were studied at birth and during the first 28 d. of life. No supplements were given following parturi-

tion. The supplements consisted of vitamin A or tocopherol or both. The tocopherol was fed daily in amounts of 0.5–1, 4, 5 or 10 g.

The prepartal supplements did not markedly affect the tocopherol levels in the serum of newborn calves before ingestion of colostrum. Tocopherol supplementation of the diet of the dams did increase the tocopherol content of the colostrum. Although there was considerable variation in individual calves, those whose dams received large tocopherol supplements had the highest serum levels of tocopherol following ingestion of colostrum. By the time calves were 28 d. of age, all calves had serum tocopherol within the same range. R. K. Waugh

**602. Pyridoxine deficiency in the calf.** B. C. JOHNSON, J. A. PINKOS and K. A. BURKE, Univ. of Ill., Urbana. *J. of Nutrition*, **40**, 2: 309–322. Feb., 1950.

Synthetic diets, with and without pyridoxine supplements, were fed to calves in order to study the requirements of calves for this vitamin. A deficiency was produced in calves fed the ration devoid of pyridoxine. Deficient calves responded to vitamin B<sub>6</sub> as pyridoxine, pyridoxal or pyridoxamine. The deficiency was characterized by anorexia, lack of growth, listlessness, dull and loose haircoat and, in some cases, by severe epileptiform fits and death. R. K. Waugh

Also see abs. no. 577.

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**603. Milking machine.** H. A. McARTHUR and J. B. DECKER (assignors to Rite-Way Products Co.). U. S. Patent 2,508,960. 11 claims. May 23, 1950. Official Gaz. U. S. Pat. Office, **634**, 4: 1241. 1950.

The milk receptacle of this pulsator-type of milker is suspended under the cow by a band running over the animal's back. Vacuum is supplied through a rubber hose. Short hoses connect the teat cups to a spout which extends from the reservoir backward under the udder.

R. Whitaker

**604. Milking apparatus.** N. CORDIS. U. S. Patent 2,509,214. 3 claims. May 30, 1950. Official Gaz. U. S. Pat. Office, **634**, 5: 1418. 1950.

A portable unit is described which consists of a truck holding several 10-gal. cans in an insulated compartment and a refrigerating unit for cooling milk as it is received from the milker and for keeping the cans of cooled milk cold.

R. Whitaker

**605. Milk weighing and recording machine.** E. C. KOSTER. U. S. Patent 2,505,552. 7 claims. April 25, 1950. Official Gaz. U. S. Pat. Office, **633**, 4: 1244. 1950.

A spring type scale for weighing pails of milk after milking is equipped with a recording device which causes a pencil to move on a record sheet. The sheet is moved stepwise with each weighing.

R. Whitaker

**606. Pressure control valve.** J. B. OLSON (assignor to James Mfg. Co.). U. S. Patent 2,506,735. 2 claims. May 9, 1950. Official Gaz. U. S. Pat. Office, **634**, 2: 449. 1950.

Water is admitted to a drinking bowl for cattle when a paddle near the bottom is depressed by the animal's nose, opening a valve. R. Whitaker

**607. Calf feeding device.** B. M. FRY. U. S. Patent 2,506,205. 3 claims. May 2, 1950. Official Gaz. U. S. Pat. Office, **634**, 1: 217. 1950.

A frame holds 2 troughs. In 1 a series of feeding nipples hang down and supply liquid feed to the calves. Over the other trough is a pipe with a series of holes on the top side. To wash the feeder the 1st trough is hinged to fold over the 2nd, and water is admitted to the spray pipe which flushes out the feed trough and nipples.

R. Whitaker

**608. Stanchion.** B. SIMONSON. U. S. Patent 2,506,112. 2 claims. May 2, 1950. Official Gaz. U. S. Pat. Office, **634**, 1: 193. 1950.

A U-shaped stanchion, hinged at the bottom and attached to the floor with a swivel, is described. A cross bar, attached to a frame over the stanchion with a swivel, completes the yoke, being easily attached to the U-shaped member by means of a key.

R. Whitaker

Also see abs. no. 551, 624.

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**609. Bulk ice cream in profit picture.** W. D. DOBSON. *Can. Dairy Ice Cream J.*, **29**, 5: 46–47, 94. May, 1950.

At the Carnation Co., bulk ice cream represents about 43% of total volume, package ice cream 31% and novelties about 26%. The gross profit on the bulk sales is slightly lower than on packaged ice cream but the difference in the margin of profit is not very great. The desire to increase the sales of bulk ice cream is not influenced adversely. There is a tendency to price bulk ice cream too low. The profit on bulk ice cream has

declined because the bulk ice cream sales have decreased. The total effect, although packaged ice cream sales have increased, has been to reduce the total volume. To increase bulk sales, the following things must be done: (a) help dealers merchandise bulk ice cream; (b) hold dealer and employee meetings and teach them how to dip bulk ice cream; (c) have dealers install separate carry-out cabinets for ice cream; (d) get the dealer, where possible, to take a lower gross margin on hand-packed ice cream to sell more product; (e) encourage sales of hand-packed ice cream. H. Pyenson

**610. Article transfer mechanism.** W. E. HEISE. U. S. Patent 2,509,565. 7 claims. May 30, 1950. Official Gaz. U. S. Pat. Office, **634**, 5: 1510. 1950.

Details are presented of a method of pushing frozen confection molds through a brine tank.

R. Whitaker

**611. Ice cream and stabilizer therefore.** S. J. WERRIN (Assignor to Stein Hall & Co.). U. S. Patent 2,502,397. 6 claims. March 28, 1950. Official Gaz. U. S. Pat. Office, **632**, 4: 1234. 1950.

Ice cream mix is stabilized by guar seed gum.

R. Whitaker

**612. What's new in the ice cream field.** G. H. WILSTER. Can. Dairy Ice Cream J., **29**: 72, 88. May, 1950.

A summary of 35 recent developments in the ice cream industry is given. H. Pyenson

Also see abs. no. 589.

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**613. Change of temperature of milk in transit from the farm to the creamery.** I. JENKINS, E. M. REEVE and A. L. PROVAN. J. Soc. Dairy Technol., **3**, 3: 182-186. Apr., 1950.

The increase in temperature of milk in cans during collection depended upon temperature of the milk when collected, air temperature during collection and time in transit. Under the conditions of the trials (summer) the average increase in temperature during collection and transit was approximately 2° F. for milk carried in open trucks and about 1° F. for milk in cans covered with a tarpulin. The authors conclude that, from the standpoint of preventing an increase in temperature of milk, no useful purpose is served by covering the cans. The solution, therefore, lies in using insulated vehicles.

E. M. Foster

**614. Milk flavors.** E. G. HOOD. Can. Dairy Ice Cream J., **29**, 4: 27-30, 56. Apr., 1950.

The author discusses the off-flavors that may be found in milk, giving the 6 following causes: (a) growth of micro-organisms, (b) feed, (c) absorbed and inhalation flavors, (d) chemical composition of the milk, (e) processing and handling and (f) enzymes and catalytic changes. Only those flavor defects grouped under (b) and (d) are present in freshly-drawn milk; the others develop after milking.

H. Pyenson

**615. Milk bottle holder.** J. G. HEUER. U. S. Patent 2,508,945. May 23, 1950. Official Gaz. U. S. Pat. Office, **634**, 4: 1237. 1950.

This holder constructed of sheet metal, for attaching to walls, is made to hold a number of milk glass bottles. The necks of the bottles are inserted in U-shaped slots in a horizontal plate.

R. Whitaker

**616. Cream separator.** C. E. DEARDORFF. U. S. Patent reissue 23,215. 15 claims. April 4, 1950. Official Gaz. U. S. Pat. Office, **633**, 1: 85. 1950.

This device consists of a disc and a handle; it is inserted into the neck of the bottle in such a manner that the disc approximately coincides with the cream line. Cream then is removed by pouring from the top of the bottle. R. Whitaker

**617. Public health milk grading a legalized illusion.** J. B. BREW, Holley, N. Y. Milk Dealer, **39**, 8: 80, 92-102. May, 1950.

The history and present status of milk grading is discussed. In producing, processing, merchandising and grading milk supplies, the author advocates that the public health official concentrate on milk. Where any sanitary milk control program is aimed directly and relentlessly at the milk itself, instead of at the barn or at the milk plant, the amount of inferior quality milk will quickly drop to, and remain at, an irreducible minimum. The dairyman who is compelled by law or other pressures to focus attention upon the details of buildings, type of milk stool, specific methods such as wiping of cows' udders before milking, and the like, is prone to think more in terms of evasions. If his attention, however, is kept upon the inherent quality of the milk he produces, he is much less inclined to take chances and will think in terms of observing those essential precautions to insure the quality of his product. C. J. Babcock

**618. Apparatus for manufacturing whipped cream.** F. F. Suellentrop (Assignor to Lemay Machine Co.). U. S. Patent 2,505,439. 6 claims.



April 25, 1950. Official Gaz. U. S. Pat. Office, 633, 4: 1214. 1950.

A device for filling the head space of cans of fluid cream with a gas, such as nitrous oxide, under pressure is described. After shaking with the gas to effect solution, the cream is converted into whipped cream when released to atmospheric pressure.  
R. Whitaker

**619. Preparation of a stabilized cream product.** L. H. CHRYSLER, and E. F. ALMY (Assignors to M and R Dietetic Laboratories, Inc.). U. S. Patent 2,503,866. 27 claims. April 11, 1950. Official Gaz. U. S. Pat. Office, 633, 2: 576. 1950.

Milk products first are subjected to a cation exchange material operating in the Na cycle. This reduces the amount of Ca to 20-70% of normal, and gives a Ca/P ratio of 1:5. The normal pH then is restored by treatment with a cation exchange material operating in the hydrogen cycle.  
R. Whitaker

Also see abs. no. 567, 570, 571, 572, 575, 597.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**620. The intermediary metabolism of the mammary gland. 1. Respiration of lactating mammary gland slices in presence of carbohydrates.**

S. J. FOLLEY and T. H. FRENCH, Natl. Inst. for Research in Dairying, Reading. Biochem. J., 45, 2: 117-125. 1949.

The respiratory metabolism of slices of mammary gland from various species was studied *in vitro*. An inverse correlation between  $Q_{O_2}$  for mammary tissue and body size is shown, being highest for the mouse and lowest for the cow. In the presence of glucose the respiratory quotient of mammary tissue from the mouse, rat, guinea pig and rabbit were greater than 1, while for ruminants (goats and cows) it was less than 1. Glucose and mannose were oxidized by lactating rat mammary gland, but galactose, lactose and fructose were not. This is in contrast to brain, retina, testis and kidney tissues which will oxidize fructose. Data are presented in 3 figures and 5 tables.  
A. O. Call

**621. The intermediary metabolism of mammary gland. 2. Respiration and acid production of mammary tissue during pregnancy, lactation and involution in the rat.** S. J. FOLLEY and T. H. FRENCH. Natl. Inst. for Research in Dairying, Reading. Biochem. J., 45, 3: 270-275. 1949.

The respiratory quotient of rat mammary tissues in glucose is below 1 at the end of pregnancy,

increases sharply at parturition and reaches a maximum of about 1.6 during lactation. Weaning brings about a decrease below 1. There is some discussion regarding the results.  
A. O. Call

**622. The effect of thyroxine and thiouracil on some of the water-soluble vitamins in milk.** (Abs.) R. CHANDA, MARY L. McNAUGHT and E. C. OWEN. Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., 45, 4: xix. 1949.

Three pairs of cows were studied for a period of 9 wk. One pair acted as a control. A second group received a 10 mg./d. subcutaneous dose of thyroxine for 3 wk. and a 3rd received 20 mg. thiouracil during the same 3-wk. test period. Thyroxine caused a decrease in phosphatase and an increase in phosphoric esters of  $B_1$ . The reverse was true when thiouracil was given. No significant differences in riboflavin were noted during treatment.  
A. O. Call

**623. Acetic acid in bovine peripheral blood and its utilization by the mammary gland.** (Abs.) G. L. McClymont, Univ. of Sydney. Biochem. J., 45, 1: i-ii. 1949.

The volatile fatty acids of bovine arterial blood were found to be over 90% acetic with small amounts of propionic, butyric and at least 2 higher acids. Expressed on the basis of mg. acetic acid/100 ml. of blood, typical ranges for arterial blood were 8-12 mg. 2-4 hr. after feeding, 3-6 mg. 24 hr. after feeding and 1.5-3 mg. 48 hr. after feeding. Both the lactating and non-lactating mammary gland removed acetic acid from the blood, leaving from 40-80%. An association was found between the decline in Reichert value of milk fat in starvation and the fall in arteriovenous difference of acetic acid.  
A. O. Call

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**624. The role of the air line hose of the milking machine in the contamination of milk.** E. S. CHURCHILL and W. L. MALLMANN, Mich. Agr. Expt. Sta., East Lansing. J. Milk & Food Technol., 13, 137-145. May-June, 1950.

Bacterial counts were determined on raw milk samples, after which each sample was laboratory pasteurized at 143° F. for 30 min., cooled and plated for thermoduric count. The dirty air line tubes were examined by pouring 20 ml. of sterile skimmilk into the hose and making total and thermoduric bacterial counts. Dirty air line hose did not appreciably increase the bacterial counts in milk collected from properly sanitized milking machines.  
H. H. Weiser

Also see abs. no. 590.

# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**625. Pathogenesis of bovine mastitis. II. The significance of hypersensitivity in streptococcal infection.** G. R. SPENCER and D. M. ANGEVINE, Wis. Agr. Expt. Sta., Madison. *Am. J. Vet. Research*, 11, 40: 317-323. July, 1950.

Normal cows were distinctly less sensitive to intradermal injections of antigens prepared from a culture of *Str. agalactiae* than were cows infected with *Str. agalactiae*. Cows with clinical mastitis had greater reactions than infected cows without clinical manifestations. Some cows with streptococcal mastitis failed to react, and irregularities in the reaction make the method of little value in diagnosis. Two formerly infected hypersensitive cows given intramammary injections of antigens developed rapid inflammatory reactions, while injections of distilled water caused no appreciable swelling. Intramammary injections of streptococcal polysaccharide also caused severe inflammatory response. Intramammary injections of polysaccharide in a normal cow produced a mild reaction similar to that following distilled water. Similar results with intramammary antigens were observed in normal and hypersensitized rabbits. These studies indicate that hypersensitivity may be an important factor in clinical bovine mastitis.

E. W. Swanson

**626. A practitioner treats mastitis.** R. CURTIS, Portage, Wis. *Vet. Med.*, 45, 7: 283-285. July, 1950.

This is a brief review of mastitis based on the experiences of a veterinary practitioner. The veterinarian is primarily interested in proper diagnosis and treatment of this disease. A differential diagnosis is essential. Various methods of treatment are discussed. Several herds of dairy cows are on an annual check basis. The cows are tested with the Hotis test and the results suggest

proper control and sanitary measures. Good herd management is one of the most valuable factors in controlling mastitis.

B. B. Morgan

**627. The treatment of bovine pyelonephritis.** E. V. MORSE, Univ. of Wis., Madison. *Vet. Med.*, 45, 5: 221-224. June, 1950.

An excellent review on the treatment of pyelonephritis in cattle is given. Several treatments are described. Until the advent of penicillin, most treatments were of doubtful value. Successful therapy depends upon early diagnosis and prompt, proper treatment. Symptomatic treatments alone, including the use of dextrose, saline and blood transfusions are ineffective. The sulfonamides have not shown much promise in the treatment of this condition when employed as the only therapy. Penicillin has given the most encouraging results. Doses of 2-3 million units have been used. Most practitioners use 10 million units of penicillin/cow. Therapy should cover an interval of about 10 d. Cows which recover clinically should be examined every 6 mo. for 18 mo. in order to determine if the animal has permanently recovered.

B. B. Morgan

**628. Sulfamethazine and blood transfusion in experimental treatment of bovine brucellosis.** R. E. WATTS, L. E. BOLEY and W. A. GREIG, III, Agr. Expt. Sta., Urbana. *Am. J. Vet. Research*, 11, 40: 304-307. July, 1950.

Repeated courses of treatment with 1.5 gr./lb. of sulfamethazine intravenously followed by 0.75 gr./lb. *per os* for 4-7 d. accompanied by a transfusion of 1 l. of citrated whole blood or 300 ml. of normal cow serum were given to brucellosis-infected cows. Three infected cows were used as controls. Changes in blood titers were insignificant and brucella were still shed in the milk of 3 of the treated cows following the experiment. One treated cow and 1 control became negative to the blood test before the end of the experiment. Death of 2 of the treated cows during

the experiment was attributed to the treatment. Reaction to the blood transfusion frequently was marked.

E. W. Swanson

**629. The treatment of retained fetal membranes and their sequelae in the bovine.** W. L. BOYD, Univ. of Minn., St. Paul. Vet. Med., **45**, 7: 263-266. July, 1950.

A brief review is presented on the various conditions of cattle in which retained fetal membranes may be involved. These included brucellosis, vibriosis and trichomoniasis. In other instances no microorganisms can be incriminated. Cows which give birth to twins frequently fail to expel the placenta. A review of the anatomy and physiology of the uterus also is presented. Symptoms, lesions and treatment of retained fetal membranes are discussed. Important sequelae of placentitis included metropéritonitis, pyometritis and abscess formation with pelvic adhesions.

B. B. Morgan

**630. The clinical use of tyrothricin-B.F.I. uterine tablets in cows.** J. L. McAULIFF, W. V. PHILLIPS and J. R. STEELE, Cortland, N. Y. Vet. Med., **45**, 6: 241, 245. June, 1950.

Tyrothricin-B.F.I. uterine tablets were used in 210 cows to prevent infections and promote healing of the uterine wall. Two to 4 tablets were inserted at each treatment. The tablet consisted of tyrothricin (0.05 g.), bismuth-formic-iodide (0.5 g.), bismuth subgallate (2.0 g.), boric acid (2.15 g.), and urea (1 g.). The cows treated were divided into 4 groups: (a) 110 retained placentas removed manually after calving, (b) 20 cows with partially removed placentas, (c) 50 cows with retained placentas after abortion and (d) 30 cows which developed metritis about 1 wk.-10 d. after calving. The results indicated that the tablets were a safe and effective material for treating retained placentas.

B. B. Morgan

**631. A quantitative study of *Trichomonas foetus* in preputial samples from infected bulls.** D. V. HAMMOND, V. R. BISHOP, G. JEFFS and W. BINNS, Utah Agr. Expt. Sta., Logan. Am. J. Vet. Research **11**, 40: 308-314. July, 1950.

Six bulls known to be infected with *T. foetus* were sampled at frequent intervals (1-2 d.) over periods as long as 6 mo. Samples of fluid were secured from the glans penis and surrounding preputial membrane by means of a glass pipette and rubber bulb. The number of *T. foetus* organisms per ml. of fluid was determined undiluted in a hemacytometer. The average collection of fluid was 0.52 ml. Of 241 examinations, 217 (90%) were positive and 3 of the bulls were positive at every examination. One bull sampled on

alternate days with pipette and swab exhibited only 6% of the organisms from the swab as found by the pipette. Wide variations in concentration of organisms were observed with each bull. The highest average was 44,000/ml. and the lowest bull averaged 80/ml. The highest single count was 488,000/ml.

E. W. Swanson

**632. Allergic response to johnin and tuberculin of various skin regions of cattle.** A. B. LARSEN, A. H. GROTH and H. W. JOHNSON, Reg. Animal Disease Research Lab., Auburn, Ala. Am. J. Vet. Research, **11**, 40: 301-303. July, 1950.

Five steers made hypersensitive to johnin and 1 hypersensitive to tuberculin were used in an experiment designed for statistical analysis to measure the reaction on various parts of the body to intradermal injections of johnin or tuberculin. The size of reaction was measured with a dermal thickness gauge. The regions in order of decreasing sensitivity were neck, back, side and caudal fold. The mean size of reaction at the neck was more than twice that at the caudal fold. Results were similar with johnin and tuberculin.

E. W. Swanson

Also see abs. no. 639.

## CHEESE

A. C. DAHLBERG, SECTION EDITOR

**633. The design and operation of the cheese trommel—its use in cheddar cheesemaking.** J. M. SHARKEY, Kraft Walker Cheese Co., Melbourne, Australia. Australian J. Dairy Technol., **4**, 1: 3-6. Jan.-Mar., 1949.

The construction details and the operation of a cheese trommel are described. The unit consists of a stainless steel perforated drum 15 ft. long and 5 ft. in diameter, with ends tapering to openings 18 in. in diameter. At about 0.2% acidity the curd with a portion of the whey is pumped into the trommel, where firming of the curd is completed. The whey drains into a specially constructed trough which conducts it to a sump vat from which the whey is pumped to separators. The trommel is mounted on rails and thus may be moved from vat to vat. The unit is claimed to be labor saving and to allow increased manufacturing output.

J. C. Olson

**634. Cheese press.** N. J. PETERS (assignor to Damrow Bros. Co.). U. S. Patent, 2,514,007. 1 claim. July 4, 1950. Official Gaz. U. S. Pat. Office, **636**, 1: 278. 1950.

To provide uniform pressure on cheese in hoops in the conventional horizontal type cheese press, a heavy coil spring is inserted between the end plate and the first hoop.

R. Whitaker

**635. Making a quality cottage cheese.** N. C. ANGEVINE, Meyer-Blanke Co., St. Louis, Mo. Milk Dealer, **39**, 9: 62, 83-89. June, 1950.

See abs. no. 557.

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**636. Production of casein yarn.** R. F. PETERSON (assignor to U. S. A.). U. S. Patent 2,512,674. 2 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635**, 4: 1104. 1950.

An alkaline solution of casein is extruded into a heated hardening bath of a metal salt and formaldehyde, followed by a final stabilization of the fibres in a concentrated buffer solution at a pH of 6-8. R. Whitaker

**637. Water paste paints.** B. O. NEWMAN (assignor to National Gypsum Co.). U. S. Patent 2,511,782 claim. June 3, 1950. Official Gaz. U. S. Pat. Office, **635**, 2: 640. 1950.

A water base paint is described, consisting of peptized casein, pigments, fillers, water and 1 of the following acids: gluconic, arabonic, mannonic, gulonic, galactonic and talonic. R. Whitaker

**638. Animal food manufacture.** R. R. HAUGH (assignor to Kraft Foods Co.). U. S. Patent 2,508,112 4 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, **634**, 3: 920. 1950.

An animal feed in the form of small pellets, made by extruding a moist plastic mass of lactose and protein feed materials is described.

R. Whitaker

Also see abs. no. 654, 655, 674.

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**639. The bactericidal effect of various disinfectants on *Str. agalactiae* on the skin and in the environment of the cow.** A. CHONKOWSKI, Vet. Lab., New Haw, Weybridge, Surrey, England. Brit. Vet. J., **106**, 5: 181-196. May, 1950.

The effect of different disinfectants at various concentrations on *Str. agalactiae* was tested. *Str. agalactiae* survived for as long as 3 wk. on various objects in the barn and up to 26 d. on the skin of cattle. The organism can multiply and persist in sores on the teats of non-infected udders, thus providing a constant source of infection. Fourteen different substances were tested *in vivo* and *in vitro*. A drug mentioned only as CTAB was found to be satisfactory. CTAB in an aqueous

solution (0.5-1%), or in cream and iodine solution was a good disinfectant for the skin, while CTAB in aqueous solution (2%) and formaldehyde were best for barns. CTAB in an aqueous solution was the most satisfactory for dairy utensils while CTAB and penicillin creams were the most efficient for the treatment of teat sores. A 0.1-0.2% CTAB aqueous solution was suggested for the routine washing of the udder before milking. B. B. Morgan

**640. On the contamination of the milk supply of the city of Pretoria with tubercle bacilli.** M. W. HENNING and W. G. VAN ASWEGEN, Inst. of Onderstepoort, Pretoria, So. Africa. J. So. African Vet. Med. Assoc., **21**, 1: 27-29 Mar., 1950.

The authors outlined a method which may be used for detection of tubercle bacilli in cows' milk. Approximately 100 ml. of each composite sample were centrifuged at 3,000 r.p.m. for 30 min. One ml. of the gravity cream from each sample was injected into separate pairs of guinea pigs. The same procedure was followed with the sediment from each sample, except the sediment was emulsified in 1 ml. of normal saline before injection. The guinea pigs were killed from 6-8 wk. following the injections and microscopic observations for tubercle bacilli were made. Of the herds producing milk for the Pretoria market, 1% produced milk contaminated with tubercle bacilli. K. M. Dunn

Also see abs. no. 625.

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**641. Method of replacing cations in milk.** R. J. MYERS (assignor to Rohm and Haas Co.). U. S. Patent 2,511,825. 3 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635**, 2: 651. 1950.

The Na of milk is exchanged for the  $\text{NH}_4$  radical by contacting it with ammonium sulphonated phenol formaldehyde cation exchange resin. The  $\text{NH}_4$  ions then are displaced by the addition of Ca and K hydroxides to the milk. R. Whitaker

**642. Détermination de l'eau incorporée au beurre par une méthode de contrôle rapide. (Determination of water incorporated in butter by a quick method.)** E. Pozzi-Escor. Lait, **20**, 295-296: 225-228. May-June, 1950

The method consists of weighing a 10-20-g. sample of butter into a graduated centrifuge tube, adding sufficient fat solvent (kerosene or gasoline are recommended) to dissolve the butterfat and

then centrifuging to obtain the aqueous layer. Readings are corrected for casein and soluble salt content and then converted to per cent moisture. The procedure is convenient and rapid. A Babcock centrifuge may be used but it is necessary to provide special cups to hold the centrifuge tubes. For production control work and most other purposes the results appear to be sufficiently accurate. S. Patton

**643. Modifications apportées a la methode de diagnostic du lait de vache dans le lait de femme. (Modifications applied to the method of detecting cow's milk in mother's milk.)** P. ROMEYER. *Lait*, 30, 295-296: 249-252. May-June, 1950.

Certain imperfections in the original method (*Lait*, 29: 576, 1949.), which is based on measurement of difference in phosphorus content of mother's milk and cow's milk, are remedied.

S. Patton

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**644. Power requirements in the churning of cream to butter by the normal buttermaking process in New Zealand.** F. H. McDOWELL, W. R. CRAIG, M. E. MARTIN and B. W. HARVEY, The Dairy Research Inst., New Zealand. *Australian J. Dairy Technol.*, 3, 4: 137-141. Oct.-Dec., 1948.

Power requirements expressed as K.W.H./ton of butter were obtained from 3 New Zealand butter plants. The requirements included power used in operating the churns during washing of butter. Average requirements in the 3 factories were sufficiently uniform to indicate that 15 units (K.W.H.)/ton of butter may be used as a working basis for calculating power costs for churning of butter in N. Z. Variations of from 10-20 K.W.H./ton of butter were observed. Most of the data were obtained from observations on 100-box capacity churns. The rate of power utilization at various stages of the churning process was studied in 1 plant. Two to 4 units/min. were required during the first 15 min., increasing to 10 at time of maximum viscosity before the breaking stage, followed by a decrease during granule formation to the time of draining. During working, the power requirements remained constant at 4.0+ units/min. J. C. Olson

**645. Diesel vs. purchased power.** R. UMBACH, R. Umbach & Assoc., Selem, O. *Milk Dealer*, 39, 9: 80-81. June, 1950.

When good diesel fuel oil could be purchased for 6¢ or less/gal., diesel power could produce a K.W.H. for less than 0.5¢ in the larger installa-

tions and for less than 1¢ in the smaller installations. The increased cost of electric power from the utilities is not as phenomenal as the increase of the price of fuel and lubricating oil and, therefore, comes close to competing with diesel power at present prices, especially in small installations from 50-500 h.p. Wasted heat recovery with the diesel and development of the gas diesel should not be overlooked. Sheer thermal efficiency is not always a sound yardstick for measuring economy of operation and the adoption of either diesel or purchased power should be closely investigated. C. J. Babcock

**646. Firetube boilers.** J. R. McDONNELL. *Operating Eng.*, 3, 6: 24-25. June, 1950.

A brief procedure for preparing a firetube boiler for boiler inspection is given. On the fireside, remove soot from the tubes, remove soot from the furnace, and inspect grates and setting. On the waterside, drain water from boiler, remove hand-hole manhole plates, remove oil and wash. Boiler accessories should be checked. H. L. Mitten, Jr.

**647. How to handle ammonia safely.** L. BRANDT, Pa. Salt Mfg. Co. *Power*, 94, 7: 85-87. July, 1950.

Safe handling of anhydrous  $\text{NH}_3$  depends on how thoroughly the handler understands the three potentially hazardous properties of the refrigerant: (a) toxicity, (b) flammability of oil-ammonia mixtures and (c) rapid expansion of liquid.

$\text{NH}_3$  odor is so irritating that no one purposely inhales dangerously high concentrations.  $\text{NH}_3$  is not cumulatively toxic. It gives warning of its presence by its irritating properties. Gas masks should be stored outside  $\text{NH}_3$  equipment rooms and be used when it is necessary to work in areas of high concentration.

Although limits of flammability are from 16-27% by volume in air, the range is so narrow that a flame cannot be made self-sustaining. Investigations of  $\text{NH}_3$  fires usually reveal that they were caused by leakage of oil- $\text{NH}_3$  mixtures rather than by  $\text{NH}_3$  alone. The high side  $\text{NH}_3$  contains oil from the compressor. When the high side of an  $\text{NH}_3$  system must be emptied, it is preferable to store the charge in another part of the system rather than to replace it in the cylinders. In case the charge is placed in cylinders, the cylinders should be carefully weighed to prevent overfilling and danger from bursting on heating.

Because  $\text{NH}_3$  attacks nonferrous alloys, the universal construction material is steel and the piping is extra-heavy steel. Valves usually are of the backseating type which can be packed under pressure. H. L. Mitten, Jr.

**648. The indicator-card story.** T. MITCHELL, Frick Co., Waynesboro, Pa. Operating Eng., 3: 36-37. June, 1950.

A method for attaching a card indicator system to large NH<sub>3</sub> and Freon compressors is suggested. The cards tell the refrigerating work being done by each cylinder. They also indicate defective operation. A number of cards are presented to show typical curves for various types of malfunction.

H. L. Mitten, Jr.

**649. Process and apparatus for cooling milk and other liquids.** G. GRINDROD. U. S. Patent 2,511,582. 19 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, 635, 2: 588. 1950.

An internal tube cooler is described in which the milk is pumped at high velocity to permit rapid cooling without freeze-on, thus permitting the use of a low temperature refrigerant. The amount of refrigerant used is adjusted automatically to compensate for variations in pressure and velocity of the milk.

R. Whitaker

**650. Ice cream freezer.** L. N. YOHE. U. S. Patent 2,511,313. 6 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, 635, 2: 519. 1950.

This invention is concerned with a means for removing separately, the front end and the cylinder or barrel of a horizontal freezer. Suitable adjustments are provided to prevent leakage at the joints and to compensate for wear.

R. Whitaker

**651. Apparatus for freezing desserts.** L. N. YOHE. U. S. Patent 2,511,314. 8 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, 635, 2: 519. 1950.

The cylinder of a horizontal freezer is extended to form an elongated tubular portion beyond the dasher; it contains a device for varying the size of the outlet from which the frozen ice cream is withdrawn.

R. Whitaker

**652. Sonic method for control of air in ice cream.** R. FRIEDMAN (assignor to Westinghouse Electric Corp.). U. S. Patent 2,508,152. 7 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, 634, 3: 931. 1950.

The overrun of ice cream is controlled automatically by varying the amount of air admitted to a continuous freezer. The overrun is measured from changes in sound velocity resulting from passing sound waves through a layer of given thickness of the ice cream as it leaves the freezer.

R. Whitaker

**653. Apparatus and method for deaeration of liquids.** W. McK. MARTIN (assignor to Schwarz

Eng. Co.). U. S. Patent 2,507,797. 10 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, 634, 3: 839. 1950.

Milk and other liquid food products may be continuously deaerated by this device, which consists of a rotor operating in a vacuum chamber. The product is introduced into the center of the rotor, whence it travels outwards past baffles that break it up into small droplets and then collect the deaerated droplets and discharge the liquid from the chamber.

R. Whitaker

**654. Apparatus and method for the evaporation of liquids.** G. G. ZAHM (assignor to Hurd Corp.). U. S. Patent 2,512,513. 6 claims. June 20, 1950. Official Gaz. U. S. Pat. Office, 635, 3: 949. 1950.

A vacuum concentrator for milk and other liquid food products has a method for collecting the condensable volatile flavor forming materials from the vapor proportionally returning them to the concentrate after pasteurization.

R. Whitaker

**655. Evaporator and separator.** R. O. HENSEZ. U. S. Patent 2,512,938. 11 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, 635, 4: 1172. 1950.

An entrainment separator for vacuum pans is described.

R. Whitaker

**656. Emulsifying apparatus.** H. S. BROCHNER (assignor to International Morfat Co.). U. S. Patent 2,509,288. 5 claims. May 30, 1950. Official Gaz. U. S. Pat. Office, 634, 5: 1438. 1950.

Stable oil-in-water emulsions or creams may be made in this equipment, which consists of a chamber in which are located 2 perforated cone-shaped nozzles pointing toward each other and separated by a distance about 1/3 of the diameter of the chamber. The continuous phase liquid is introduced under pressure through the lower nozzle, the dispersed phase liquid through the upper nozzle. The emulsion leaves the chamber through an outlet in the top.

R. Whitaker

**657. Apparatus for heat-treating and stabilizing liquid food products.** C. O. BALL (assignor to Owens Illinois Glass Co.). U. S. Patent 2,508,212. 11 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, 634, 3: 948. 1950.

Milk and other liquid food products may be heated continuously in this equipment, consisting of a 3-compartment cylindrical chamber containing steam under pressure. The liquid to be heated passes through a spiral channel located in the wall of the cylinder.

R. Whitaker

**658. Art of pasteurizing milk, etc.** G. H. BROWN (assignor to Radio Corp. of America.). U. S. Patent 2,510,796. 4 claims. June 6, 1950. Official Gaz. U. S. Pat. Office, **635**, 1: 280. 1950.

Milk is pasteurized by the high-temp., short-time method by passing through a high frequency electric field. The hot milk is rapidly cooled by spraying into a vacuum chamber. The hot vapors are employed to heat the incoming cold raw milk.

R. Whitaker

**659. Pumping system for milk processors.** R. H. STEINBERG and H. COHEN. U. S. Patent 2,512,045. 1 claim. June 20, 1950. Official Gaz. U. S. Pat. Office, **635**, 3: 829. 1950.

In a milk pasteurizing unit containing a milk to milk regeneration step, an auxiliary milk pump is provided ahead of the raw milk inlet to maintain milk pressures throughout the system within a carefully selected range. The system is electrically controlled and operated.

R. Whitaker

Also see abs. no. 633, 634, 672.

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**660. Efficient mechanical milking.** W. G. WHITTLESTON, Dept. of Agr., Hamilton, New Zealand. Australian J. Dairy Technol., **3**, 2: 45-72. Apr.-June, 1948.

This is a review article to acquaint practical workers in the dairy industry with the theory and practice of machine milking. There are 3 parts: Part I, "The cow," in which milk let-down, reflexes, stimulation, stripping, milking machines and mastitis, and speed of milking are discussed; Part II, "The milking machine," presenting pumps, pulsators, relief valves, teat cups and claws, and rubber ware; and Part III, covering the various aspects of installation and servicing.

J. C. Olson

**661. Claw for milking machines.** G. H. GASCOIGNE (assignor to Gascoignes, Ltd.). U. S. Patent, 2,507,969. 3 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, **634**, 3: 883. 1950.

This manifold for milking machines has 4 openings for teat cups, so placed as to permit easy attachment to the udder. A finger-operated valve cuts off the vacuum and milk outlet and vents the manifold for removal from the udder.

R. Whitaker

**662. Teat cup holder for milking machines.** C. B. FINN. U. S. Patent 2,512,926. 3 claims.

June 27, 1950. Official Gaz. U. S. Pat. Office, **635**, 4: 1169. 1950.

A holder for positioning teat cups under the cow's udder is described.

R. Whitaker

**663. Flow indicator for milking machines.** L. DINESEN (assignor to Perfection Mfg. Co.). U. S. Patent 2,513,627. 2 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636**, 1: 179. 1950.

An attachment for a milking machine, inserted in the lines leading from the teat cups, indicates when milk is flowing through the lines to the milk-collecting reservoir.

R. Whitaker

**664. Milking machine.** E. T. JANSSON (assignor to Akicbolaget Separator Corp.). U. S. Patent 2,510,581. 4 claims. June 6, 1950. Official Gaz. U. S. Pat. Office, **635**, 1: 225. 1950.

A vacuum-operated pulsating type of milker is constructed as part of the lid of a milk pail.

R. Whitaker

**665. Means for milking and handling the milk of farm animals.** G. R. DUNCAN. U. S. Patent 2,512,094. 13 claims. June 20, 1950. Official Gaz. U. S. Pat. Office, **635**, 3: 841. 1950.

A milking parlor arrangement, including a system of entrance and exit for the animals, a 2-cow milking platform with the animals standing tail to tail and provisions for cooling and storing the milk is described.

R. Whitaker

**666. Cow tail holder.** A. J. KIENE. U. S. Patent 2,513,494. 2 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636**, 1: 144. 1950.

A cow is prevented from swishing her tail by this device which grips the tail and one rear leg.

R. Whitaker

**667. Self-cleaning drinking bowl for animals.** W. H. SHELDON (assignor to Michigan State Board of Agr.). U. S. Patent 2,513,753. 5 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636**, 1: 213. 1950.

A siphon arrangement flushes out this animal drinking bowl as soon as the animal's nose ceases to press a lever in the bowl.

R. Whitaker

**668. Stock feeding appliance.** J. A. POWELL. U. S. Patent 2,512,260. 2 claims. June 20, 1950. Official Gaz. U. S. Pat. Office, **635**, 3: 884. 1950.

A feeding trough for cattle consists of a cylindrical reservoir holding dry feed and a feeding trough protected by an overhang to prevent water from collecting in the trough. A mechanism in the trough, actuated by the animal's nose, agitates the feed in the reservoir to keep it uniform and

conveys it from the reservoir into the trough as it is consumed.

R. Whitaker

**669. Comparative evaluation of rotenone formulations for cattle grub control.** J. R. DOUGLAS and D. P. FURMAN, Univ. of Cal. at Davis and Berkeley. *J. Econ. Entomol.*, **42**, 6: 884-887. Dec., 1949.

Nine spray formulations were applied by power sprayer at 250-400 lb. pressure, and compared in efficiency of cattle grub (*Hypoderma lineatum* and *H. bovis*) control. About 2 qt. of spray/animal were used, and control data were determined 7 d. after treatment.

A wetting agent increased the effectiveness of ground derris formulations but sulfur did not. Five lb. of derris (5% rotenone content) to 100 gal. of water was about half as effective as a 10-lb. rate. Piperonyl butoxide and N-octyl bicycloheptene dicarboximide did not increase rotenone efficiency. *H. bovis* was more resistant to insecticides than *H. lineatum* E. H. Fisher

**670. La brebis productrice de lait et facteur économique.** (The sheep as a producer of milk and an economic factor.) V. DE SA. *Lait*, **20**, 295-296: 245-248. May-June, 1950.

It is contended that sheep could be better utilized as producers of milk than is presently the case. The point is made that wool production is not antagonistic to milk production in the sheep and that high production of wool and milk usually go hand in hand. Recommendations are made concerning methods for making better use of the sheep as a milk producing animal.

S. Patton

Also see abs. no. 686, 687, 688.

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**671. Edible food product.** A. RUBIN. U. S. Patent 2,511,082. 2 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635**, 2: 459. 1950.

An ice cream novelty consisting of a split doughnut and ice cream is described. One half the doughnut is placed flat side down on the bottom of a round package of approximately the same diameter. Ice cream then is filled into the package and the remaining half doughnut, also flat side down, is placed on top. The ice cream fills the holes in the doughnut halves.

R. Whitaker

**672. Measuring dispenser for filling ice cream containers and the like.** K. P. HERBOLD (assignor to Eskimo Pie Corp.). U. S. Patent 2,510,-

576. 7 claims. June 6, 1950. Official Gaz. U. S. Pat. Office, **635**, 1: 223. 1950.

A device is described for delivering a measured quantity of ice cream into a container, using a reciprocating piston, the volume of which may be easily changed manually to compensate for changes in overrun

R. Whitaker

**673. Coin-freed ice cream vending machine.** W. H. PARTRIDGE. U. S. Patent 2,511,076. 5 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635**, 2: 458. 1950.

A vending machine for wrapped pieces of ice cream, cooled by air refrigerated by a small motor driven compressor, is described.

R. Whitaker

Also see abs. no. 650, 651, 652.

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**674. What to do with surplus milk.** D. ARMERDING, Mojonner Bros. Co., Chicago, Ill. *Milk Dealer*, **39**, 9: 68-69. June, 1950.

The dairy industry should take care of its own surpluses by enriching its own products instead of expecting the baking industry and others to use its surplus milk powder. Increasing the serum solids content of the fluid milk consumed annually from 8.5-10% automatically would remove 750 million lb. of nonfat dry milk solids from the market. This enrichment would increase the nutritional ingredients by 12.5% with only a 5% increase in selling price. Increasing the solids-not-fat in evaporated milk from 18-19% would absorb another 27-30 million lb. of nonfat dry milk solids and the serum solids of other products could be increased.

C. J. Babcock

**675. La méthode de controle et de conservation du lait maternel au lactarium.** (Quality control and preservation of mother's milk at the "lactarium.") A. ROISSIER and J. BERTRAND. *Lait*, **20**, 295-296: 252-256. May-June, 1950.

Methods of determining acidity, bacteria count, density, fat, solids-non-fat, watering and adulteration of mother's milk are given. Procedures for bottling, sterilizing and refrigerating the milk also are presented.

S. Patton

**676. Bottle crate.** H. KERSHAW. U. S. Patent 2,512,096. 2 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635**, 4: 1212. 1950.

A milk bottle crate, having metal bound wooden sides and metal rod partitions, is described.

R. Whitaker



**677. Milk bottle carrying case.** E. R. ERICKSON (assignor to C. E. Erickson Co.). U. S. Patent 2,512,855. 6 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635**, 4: 1151. 1950.

An all metal milk bottle crate is described.

R. Whitaker

**678. Pasteurizer.** E. K. MALME (assignor to Guard-It Mfg. Co.). U. S. Patent 2,513,577. 6 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636**, 1: 165. 1950.

A few gallons of milk in a can may be pasteurized automatically by placing the can in this electrically heated and agitated farm or home pasteurizer.

R. Whitaker

**679. Rapport over de Zuivelindustrie in de U.S.A. (Report on the dairy industry in the U.S.A.).** Publication of the General Netherlands Dairy Union of Cooperative factories, The Hague, Holland. 78 pp. 1949.

Report of a Dutch Committee which visited the U.S.A. in April, 1949. A. F. Tamsma

Also see abs. no. 649, 653, 658, 659.

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**680. The paralytic action of histamine on the ruminal musculature.** R. CLARK, Inst. of Onderstepoort, Pretoria, So. Africa. J. So. African Vet. Med. Assoc., **21**, 1: 13-15. Mar., 1950.

Merino sheep with permanent ruminal fistulas were used for the trials. The ruminal cavity was connected to a rubber diaphragm tambour for the registration of pressure changes.

Complete rumen stasis followed the intravenous injection of 1-2 mg. of histamine. This cessation of ruminal movement lasted for a period of 30 min. The sheep defecated repeatedly from 5-10 min. following the histamine administration, indicating a constriction of the intestines. The ruminal stasis and intestinal constriction were prevented or cured by the administration of anti-histamine drugs prior to or following the histamine administration.

The authors point out that their findings may give a physiological basis for the use of anti-histamine drugs for the treatment of various types of bloat in ruminants. It was shown that the rumen paralyzed with histamine was still capable of responding to faradic stimulation of the vagus nerve. This response showed that the paralysis caused by the histamine was of myogenic origin. K. M. Dunn

**681. Purification of hyaluronidase.** H. TINT and R. BOGASH, Wyeth Inst. Applied Biochem., Philadelphia, Pa. J. Biol. Chem., **184**, 2: 501-509. June, 1950.

The effects of variations of pH, ionic strength and alcohol concentration on the solubility of hyaluronidase and their influence on purification procedures were studied employing a crude hyaluronidase obtained by extracting decapsulated, ground bovine testes in the cold with acetic acid followed by precipitation with  $(\text{NH}_4)_2\text{SO}_4$ . Optional recovery and purity of hyaluronidase were obtained in the pH range of from 6-8, 0.15 ionic strength and ethanol concentration between 20-40%. Refinement of the single stage ethanol precipitate by 1 salting-out operation with  $(\text{NH}_4)_2\text{SO}_4$  brought 50-60% of the recovered solids to a purity of about 40 units/mg. The over-all recovery of starting material obtained was above 30% with a 20-fold increase in activity.

H. J. Peppler

**682. The competitive interaction of organic anions with bovine serum albumin.** F. KARUSH, Sloan-Kettering Inst. for Cancer Research, N. Y. J. Am. Chem. Soc., **72**, 6: 2714-2718. June, 1950.

The competitive binding by bovine serum albumin of the detergent sodium dodecyl sulfate and dye p-(2-hydroxy-5-methylphenylazo)-benzoic acid was investigated to determine the relative heterogeneity of the binding sites. Interpreting the data in terms of a comparison of self-competition *vs.* detergent competition, it was concluded that the group 1 sites, which bind the dye most strongly, also bind the detergent most effectively and are able to assume structures complementary to a wide range of configurations. Group 2 sites bind less strongly and are more restricted in the range of configurations assumed.

H. J. Peppler

**683. Heterogeneity of the binding sites of bovine serum albumin.** F. KARUSH. Sloan-Kettering Inst. for Cancer Research, N. Y. J. Am. Chem. Soc., **72**, 6: 2705-2713. June, 1950.

The binding of the anionic dye p-(2-hydroxy-5-methylphenylazo)-benzoic acid by bovine serum albumin was studied at 5 and 25° C. over a wide range of concentrations. An assumption of 22 binding sites/protein molecule affords accurate description of the data. The dye binding sites can be divided into 2 groups: Group 1 contains between 4 and 5 sites and is characterized by a high binding constant; Group 2, with about 17 sites, has a relatively low binding constant. The ability of serum albumins in solution to exist

in many molecular configurations of approximately equal energy is discussed. An hypothesis of configurational adaptability has been advanced to account for the distinctive binding properties of serum albumins. H. J. Pepper

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**684. An evaluation of hypochlorites and cleaner-sanitizers.** N. E. LAZARUS, Lazarus Lab., Buffalo, N. Y. Milk Dealer, 39, 9: 50-59. June, 1950.

The relatively low cost, rapid action and wide effectiveness at high dilutions are some of the advantages provided by hypochlorites; if they are used properly on organically clean surfaces, they are effective chemical sanitizing agents. Testing methods for hypochlorites are given. The favorable qualities of quaternary ammonium compounds are summarized as follows: (a) not affected by extremely high temperatures and efficient down to 50° F.; (b) nontoxic, nonirritating, stable, colorless, odorless and nonvolatile in use; (c) neither a primary irritant nor a sensitizer when applied to the skins of humans or animals in a dilution as strong as 1:1,000; (d) exhibit good germicidal properties in the presence of large amounts of organic matter, such as horse-serum, skimmilk and ice cream mix; (e) contain no mercury compounds, hypochlorites, phenols, or formaldehyde, hence are not classified by FDA as a poison when used as recommended dilutions as specified by the manufacturers; (f) relatively constant in their rate of kill and on short exposures will destroy over 95% of vegetative cells in concentration as great as 1:15,000; (g) exhibit desirable qualities of surface activity, hence have greater penetration; (h) generally, extremely selective for Gram-positive bacteria, particularly thermotolerants and, to a slightly less degree, for Gram-negatives; (i) long residual action which is essential in practical operation. Testing methods are given. The use of cleaner-sanitizers is a step in the right direction for securing better results with lower operating costs, labor and time. C. J. Babcock

**685. Sanitation in the food industries, with special reference to chemical cleaners and sanitizers.** C. G. DUNN, Mass. Inst. of Technol., Cambridge. Wallerstein Lab. Comm., 13, 41: 121-140. June, 1950.

In addition to reviewing the principles of cleaning and sanitizing operations in food plants, the more common detergents and sanitizing agents

are classified and their roles are described briefly. Some applications of detergents and chemical sanitizers are cited; bibliography of 136 references is provided. H. J. Pepper

**686. Comparative effectiveness of DDT, methoxychlor and dichlorodiphenyl dichloroethane residues against house flies and Aedes floodwater mosquitoes.** A. R. ROTH and A. W. LINDQUIST, U.S.D.A., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 6: 871-873. Dec., 1949.

Laboratory tests were made with acetone solutions of 3 insecticides to determine their effectiveness in knockdown and kill of the house fly when used at concentrations of from 1-80 mg. of toxicant/ft.<sup>2</sup> with exposures of from 1-10 min. At low exposure periods with low dosage of toxicant, DDT was slightly more toxic than dichlorodiphenyl dichloroethane and several times as toxic as methoxychlor. Higher toxicant dosages plus longer exposure periods eliminated these differences in toxicity. E. H. Fisher

**687. Effect of temperature on knockdown and mortality of house flies exposed to residues of several chlorinated hydrocarbon insecticides.** R. A. HOFFMAN and A. W. LINDQUIST, U.S.D.A., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 6: 891-893. Dec., 1949.

Laboratory tests with the house fly compared the residues of several insecticides at relatively low to high dosages, exposure periods and temperatures. DDT, DDD and methoxychlor caused faster knockdown and greater kill at 70° F. than at 90° F. Heptachlor, parathion, chlordane, dieldrin and toxaphene were more effective at 90° F.

It was surmised that the house fly is controlled with low dosages of DDT in cool climates, whereas a higher dosage is needed where it is warm. E. H. Fisher

**688. Development of resistance to organic insecticides other than DDT by house flies.** R. B. MARCH and R. L. METCALF, Univ. of Cal., Riverside. J. Econ. Entomol., 42, 6: 990. Dec., 1949.

Field and laboratory tests with the house fly revealed that flies resistant to DDT also may be resistant to each benzene hexachloride and dieldrin. There is no evidence that resistant fly strains will become susceptible after non-exposure to the insecticide for several generations.

Presently accepted fly control procedures with residual insecticides may need to be revised with emphasis on sanitation, use of repellents and space sprays. E. H. Fisher



# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the  
International Association of Ice Cream Manufacturers  
and the Milk Industry Foundation

### BOOK REVIEW

**689. Annual Review of Biochemistry, vol. XIX.** J. M. LUCK, editor. Annual Reviews, Inc., Stanford, Calif. 596 pp. \$6.00. 1950.

The topics covered are: Biological oxidations, V. R. Potter; Proteolytic enzymes, M. Laskowski; Nonoxidative, nonproteolytic enzymes, P. P. Cohen and R. W. McGilvery; Carbohydrate chemistry, M. L. Wolfram and J. M. Sugihara; Chemistry of lipids, H. J. Deuel, Jr.; Chemistry and metabolism of the steroid hormones, G. Pincus; Chemistry of amino acids and proteins, R. K. Cannan and M. Levy; Nucleic acids, purines and pyrimidines, G. Schmidt; Carbohydrate metabolism, S. Ratner and E. Racker; Fat metabolism, G. Medes; Metabolism of proteins and amino acids, P. P. Swanson and H. E. Clark; Chemistry of the hormones, A. White; Water-soluble vitamins, E. E. Snell and L. D. Wright; Fat-soluble vitamins, T. Moore; Nutrition, H. M. Sinclair; Muscle, F. B. Straub; Biochemistry of neoplastic tissue, C. Carruthers; Chemical composition of blood plasma and serum, H. A. Krebs; Pyrrole pigments, R. Lemberg and J. W. Legge; Immunochemistry, P. Grabar; Biochemistry of antibiotics, H. E. Carter and J. H. Ford; and Partition chromatography, A. J. P. Martin.

As is usual in this series of publications, the authors have restricted their coverage somewhat more than the title would indicate in a number of instances. The reviews in general cover the material appearing during 1949, although earlier material is included in a number of cases where a topic has not been reviewed regularly.

The reviews on the whole are up to the high standards which one has come to expect of this publication. The subject and author indices are adequate. This is a very valuable reference book for any one working in the more fundamental areas of dairy science, as well as for others in biology and biochemistry. F. E. Nelson

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**690. Ketosis and the dairy cow.** C. B. KNOTT. Penn. State College, State College. Milk Plant Monthly, 39, 8: 76-77. Aug., 1950.

Ketosis and its relation to "cowy" flavor in milk is discussed. Symptoms and methods of treating the disease also are included.

J. A. Meiser, Jr.

**691. Some clinical uses of a new antihistamine.** G. R. MOORE, Mich. State College, East Lansing. Vet. Med., 45: 328-329. Aug., 1950.

A brief report is presented on the use of a new antihistamine drug (dimethylaminooctoxymethylbenzyl-pyridine succinate) in the treatment of livestock for certain animal diseases. Two of 3 cases of bovine stomatitis showed improvement, also 3 out of 6 cases of chronic calf scours. Four cases of persistent bloat were treated and the antihistamine therapy was of value. Three cases of bloat in 5-7-mo.-old calves were relieved by a single intravenous injection. Symptoms were relieved in cases of retained placenta and respiratory infections in cattle. B. B. Morgan

**692. Occurrence of the ear mite, *Railletia auris* (Leidy, 1872), of cattle in Colorado.** O. W. OLSON and F. K. BRACKEN. Colo. State College Fort Collins. Vet. Med., 45: 320-321. Aug., 1950.

This is the first report of ear mites (*R. auris*) in cattle since the original description by Leidy in 1872. The mites were found in the tympanic membrane. Clinical symptoms included obvious distress, the head was held in an extended position and twisted to one side to give the impression that the animal was listening to sounds near the ground. Other symptoms were emaciation

and anorexia. The animal was sacrificed and at autopsy mites were recovered from the bulla tympanica.  
B. B. Morgan

**693. Veterinarians' role in public health.** J. H. STEELE, U. S. Public Health Service, Atlanta, Ga. *Vet. Med.*, 45: 311-312. Aug., 1950.

The role of the veterinarian in the safeguarding of human health by the control of animal diseases is reviewed. Important diseases mentioned include bovine tuberculosis, brucellosis, rabies, trichinosis, Q fever and swine influenza. Brucellosis is still the main occupational disease of veterinarians. Only milk from brucellosis-free herds should be permitted to be sold as grade A.  
B. B. Morgan

## BUTTER

O. F. HUNZIKER, SECTION EDITOR

**694. Mottle in butter.** L. L. MULLER, Dept. of Agr., Brisbane, Australia. *Australian J. Dairy Technol.*, 4, 1: 7-8. Jan.-Mar., 1949.

A special microscopic technique for observation of moisture droplets in butter is described. Butter is smeared between a slide and coverslip, the latter supported by 2 strips of gold leaf cemented to the slide. A chamber approx.  $30\mu$  in depth thus was obtained and served to reduce materially distortion of the droplets. A photomicrograph of a portion of a deep yellow patch of mottled butter is shown which clearly depicts large water droplets containing undissolved salt crystals. The contrast between these and normal-sized water droplets also may be observed. The factors governing mottling in butter are reemphasized.

J. C. Olson

**695. Plant and equipment sanitation in relation to butter quality.** E. G. HOOD. *Can. Dairy Ice Cream J.*, 29, 6: 27-31. June, 1950.

The most commonly encountered flavor defects recorded in Canadian butter were: surface taint, fishy tendency, tallowy or oxidized, rancid, surface flavor, unclean off-flavors, stale, yeasty, barny and malty. Mold and bacterial surface discoloration also has been common. The majority of defects were found to be associated with the microbiological flora of the butter. Experimental work has shown that recontamination from equipment after pasteurization may completely offset the beneficial effects of pasteurization. Other factors affecting the growth of bacteria in butter are temperature of holding, salt content, extent of working the butter, care of equipment, care of churn, sterilization treatment, air contamination, purity of wash water and general plant sanitation.  
H. Pyenson

**696. Variable speed churn and butter worker.** F. G. CORNELL, JR. (assignor to General Dairy Equipment Co.). U. S. Patent 2,514,375. 9 claims. July 11, 1950. *Official Gaz. U. S. Pat. Office*, 636, 2: 459. 1950.

A cube-shaped churn with the corners cut off, rotates about a diagonal axis. Access to the churn is through a manhole in 1 side.

R. Whitaker

**697. Questions and answers about the water insoluble acids and butyric acid tests for butter and cream.** W. H. MARTIN and T. J. CLAYDON, Kansas State College, Manhattan, R. ALBERTS, Am. Butter Inst., A. C. KEITH, Latimore Lab., Topeka, Kansas. *Butter, Cheese & Milk Prod. J.*, 41, 7: 22-23, 58-60. July, 1950.

A panel of experts has answered 28 of the most common inquiries concerning the W.I.A. test and butyric acid test to determine the quality of cream and butter. The discussion considers methods, reliability and interpretation of the results of the test.  
H. E. Calbert

**698. Margarine sales cutting producer income.** J. S. TURNBULL. *Can. Dairy Ice Cream J.*, 29, 39-40, 42. June, 1950.

The Canadian government is faced with the task of attempting to protect the producer of butterfat through the medium of a floor under creamery butter. The author takes the view that practical controls over the manufacture and sale of margarine would be sufficient to protect the butter producer. Attempts should be made to divert milk production to other manufactured products. The consumer demand for butter should be increased.  
H. Pyenson

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**699. Use and properties of non-fat dry milk solids in food preparation. I. Effect on viscosity and gel strength.** LURA M. MORSE, DOROTHY S. DAVIS and E. L. JACK. U. of Cal., Davis. *Food Research*, 15, 3: 200-215. May-June, 1950.

Use of dry milk in baked products primarily from the standpoint of effect on body and viscosity of batters, doughs and pastes is reported. Dry milk increases gel strength and viscosity of mixtures in proportion to amounts added, but compensatory adjustments may be made by reducing the amount of flour used. Line graphs are given from which body and viscosity values may be predicted. Sugar decreases gel strength as a rule

but increases viscosity. Fat and salt have lesser effects in somewhat the same directions. Decreases in pH lower the viscosity and gel strength, often curdling thin pastes, but pH increases up to pH 8.0 exhibit inconsistent results. F. J. Doan

**700. Use and properties of non-fat dry milk solids in food preparation. II. Use in typical foods.** LURA M. MORSE, DOROTHY S. DAVIS and E. L. JACK. U. of Cal., Davis. Food Research, 15, 3: 216-222. May-June, 1950.

The optimum quantities of dry milk are determined for use in such foods as cream soups, custards, white sauces, chocolate puddings, egg noggs, waffles and angel, butter-sponge and plain butter cakes. Dry milk can be used to advantage in each case for improving both nutritional quality and palatability of the product. F. J. Doan

**701. Use of whey in bakery goods.** L. V. ROGERS, Dairy Prod. Research Lab. U.S.D.A. Natl. Butter & Cheese J., 41, 3: 31. Mar., 1950.

Since fluid whey contains half the solids of the milk, it is desirable from the nutritional standpoint to use it in the production of foods. Either fresh fluid, condensed or dried whey can be used in the production of sherbets, cheese foods and bakery goods. Doughs using whey produce the more tender cakes and cookies. Doughnuts made with whey remain soft and retain their good eating qualities for a longer period. The milk sugar of the whey aids in the development of a uniform brown crust on baked goods. Formulas for the use of whey in baked goods can be obtained from the Division of Dairy Products Research Laboratories, Bureau of Dairy Industry, U.S.D.A., Washington 25, D. C. H. E. Calbert

**702. Consumer acceptance of bread containing different amounts of nonfat dry milk solids.** E. L. JACK, Univ. of Cal., Davis. Butter, Cheese & Milk Prod. J., 41, 5: 27. May, 1950.

Breads containing 0, 6, 10 and 14% nonfat dry milk solids were fed to a group of 320 boys ages 8-16 yr. A bread containing each level of nonfat dry milk solids was fed for a period of 8 wk. until the cycle was completed. The 8-wk. intervals were varied to eliminate any seasonal variations that might influence bread consumption. As the amount of nonfat dry milk solids in the bread increased, the bread consumption also increased. The increases in bread consumption averaged as follows: 6% level, consumption increased 4.4%; 10% level, consumption increased 7.1%; 14% level, consumption increased 12.6%. H. E. Calbert

**703. Studies on the reconstitutability of whole milk powder.** U. S. ASHWORTH and R. HIBBS. Can. Dairy Ice Cream J., 29, 6: 84-86. June, 1950.

There is strong indication that the typical powdered milk flavor is one of the effects of slight insolubility. Stale flavor can be shown to be associated with the development of insolubility during storage. Whole milk powder evaporated to 40% T.S. is more soluble than whole milk powder evaporated to 20% T.S. before drying. None of the wetting agents used were very successful in improving the rate of dispersion of a typical spray-dried whole milk powder. Other studies show that the rate of dispersion of lactose and protein are similar. H. Pynson

**704. Mixing device.** G. D. TURNBOW and A. V. OSBORNE (assignors to Chester-Jensen Co.). U. S. Patent 2,513,382. 3 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, 636, 1: 115. 1950.

This device was designed to reconstitute powdered milk with water in commercial quantities without making a foam. The powdered milk is metered from a supply tank erected above the mixing vat and flows downward into a cylinder partially submerged in the water in the mixing vat. A rotating agitator revolves in the cylinder, drawing water in through ports, suspending the powder and forcing the reconstituted milk out a lower set of ports into the mixing vat. A propeller-type agitator in the vat insures uniformity of the mixing vat contents. R. Whitaker

**705. How to prepare and store skim milk for use in ice cream.** B. H. WEBB, U.S.D.A. Am. Milk Rev., 12, 2: 46-49. Feb., 1950.

Skim milk may be stored as plain condensed, superheated condensed, frozen condensed, sweetened condensed, low lactose condensed and dried. Detailed processing procedures are omitted but attention is called to the importance of the effect of temperature on viscosity and protein stability as these in turn influence the storage life of the product. D. J. Hankinson

**706. Expanding the market for non-fat dry milk solids.** B. W. FAIRBANKS. Can. Dairy Ice Cream J., 29, 6: 52-56. June, 1950.

Three ways open in 1950 to expand domestic market for non-fat dry solids are to increase commercial exports, to increase sales in domestic market and to continue to sell to the government under the price support program (it may not last). H. Pynson

**707. Milk powder industry sins source of manufacturers' problems.** H. LAMARCHE. *Can. Dairy Ice Cream J.*, 29, 6: 80-82. June, 1950.

The home and abroad competition would be easy to meet and there would be no more overproduction of milk powder if the cost of production was controlled, the quality of the product was improved and the distribution was made more adequate.  
H. Pynson

**708. Apparatus for turning milk.** M. E. LAWRENCE. U. S. Patent 2,511,643. 1 claim. June 13, 1950. *Official Gaz. U. S. Pat. Office*, 635, 2: 604. 1950.

A small insulated box holds a can of milk for the purpose of producing a desirable clabber on souring. The temperature is maintained automatically at 85° F. by a thermostatically-operated electric light bulb situated below the can.

R. Whitaker

Also see abs. no. 758.

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**709. Effect of reducing substances on starter culture action in agitated and unagitated milk.** H. KATZNELSON and E. G. HOOD. *Can. Dairy Ice Cream J.*, 29, 7: 27-28. 1950.

Glutathione functions as a hydrogen donor and serves primarily to poise the oxidation-reduction potential in agitated milk at a level which permits normal development of the lactic streptococci. Cysteine and glutathione stimulated acid production by a starter culture in 24-hr.-old pasteurized milk which had received some agitation, but only glutathione caused an increase in the unagitated milk with the 5 starter cultures tested; the 4 consistently produced lower acid in agitated milk, an effect which was overcome by glutathione; a single strain starter was not affected appreciably by aeration. Starter action in unagitated pasteurized milk, 24-hr.-old, was stimulated by glutathione, the effect being even more marked when the milk was agitated. Glutathione exerted its effect when added at the beginning of incubation of inoculated milk but not when added 2 hr. later.

H. Pynson

**710. Preparing and maintaining good cultures.** N. C. ANGEVINE, Meyer-Blanke Co., St. Louis, Mo. *Milk Dealer*, 39, 10: 58, 62-68. July, 1950.

See abs. no. 414.

**711. Penicillin in milk.** W. A. KRIENKE, Fla. Agr. Expt. Sta., Gainesville. *Am. Milk Rev.*, 11, 12: 24-25. Dec., 1949.

See abs. no. 152.

**712. Effects of various "drugs" in milk from mastitis-treated cows on acid production by lactic starters.** W. A. KRIENKE, Fla. Agr. Expt. Sta., Gainesville. *Milk Dealer*, 39, 7: 50, 72-75. Apr., 1950.

Whether unheated or heated as high as 241° F. for 15 min., milk containing 5 ml. of a 25% solution of sulfamethazine/100 ml. permitted practically no acid production by a buttermilk culture during 18 hr. incubation at 70° F. When the drug was present in the amount of 1 ml. of 25% solution/100 ml. of milk, there was some acid development, which in the milk pasteurized at 143° F. for 30 min. was only slightly above 0.35% titratable acidity. Reduction to 0.1 ml. of 25% solution/100 ml. of milk permitted acid development to 0.46% compared to 0.71% for the control.

After incubation for 18 hr. at 70° F. or for 7 hr. at 95° F., there was practically no acid production when the milk containing 0.0005 mg. of aureomycin hydrochloride/ml. had been pasteurized at 143° F. for 30 min. When the concentration of the "drug" was reduced to 0.00005 mg./ml. of milk, acid production was nearly normal as compared to that of the control samples. At 95° F., a sample containing an intermediate concentration of the aureomycin hydrochloride gave considerably less acid production than did the control. These results emphasize the necessity of eliminating milk containing aureomycin from the supply to be used for cultures and fermented dairy products.

C. J. Babcock

**713. Some recent advances in the bacteriology of pasteurized milk.** E. B. ANDERSON and L. J. MEANWELL, United Dairies, Ltd., Central Lab., London. *J. Sci. Food & Agr.*, 1, 3: 77-80. Mar., 1950.

During 1 yr., 18,411 samples of raw milk were examined for methylene blue reduction time and for keeping quality of the corresponding laboratory-pasteurized milk. The keeping quality was measured in terms of the per cent titratable acidity (as lactic acid) after incubation at 26° C. for 24 hr. A direct relationship was found between reduction time of raw milk and keeping quality of the corresponding pasteurized milk. Raw milk collected at atmospheric temperatures above 21° C. gave, on the average, poorer pasteurized milk-keeping quality than raw milk collected at lower temperatures, even when the raw milk quality as measured by methylene blue was the same. With bulked milk drawn from farms where utensil sterilization was practised, the keeping quality of the corresponding pasteurized milk was greater throughout the year than that of

bulk milk drawn from farms where the practice of utensil sterilization was questionable.

E. B. Collins

**714. A comparative study of presumptive media for the coliform group.** H. J. FOURNELLE and H. MACY, Univ. of Minn., St. Paul. *Am. J. Pub. Health*, **40**, 8: 934-942. Aug., 1950.

A study was made of certain media, including brilliant green bile broth, formate ricinoleate broth, violet red bile agar, desoxycholate agar and desoxycholate lactose agar, to determine which would support the best growth of coliform organisms. In 15 comparative tests, where these media were inoculated with pure cultures of coliform organisms, the counts obtained with both the liquid and solid media agreed fairly closely for any 1 culture. An equal number of trials were made in which the media were inoculated with samples of milk and cream positive for the coliform organisms. Results obtained in this latter series of tests varied from close agreement to wide differences with the same sample. A definite order of productiveness could not be determined for the liquid and solid media together in either series of tests, because of the variable results. Desoxycholate agar seemed to be the least productive of the media studied, although in most instances the differences were not great.

The bacteriostatic effect of the dyes used in these media also was checked. Brilliant green was slightly more inhibitory than crystal violet, although the effective concentrations of both were considerably greater than that used in the media. Sodium ricinoleate showed little bacteriostatic action in a concentration of less than 1:25. Sodium desoxycholate was inhibitory in concentrations of less than 1:50 up to 1:1,000. This latter concentration is customary in desoxycholate agar.

D. D. Deane

**715. Coliform, their significance and control in ice cream making.** G. W. SHADWICK, Beatrice Foods Co., Chicago, Ill. *Am. Milk Rev.*, **11**, 12: 40-41. Dec., 1949.

See abs. no. 441 (1949).

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**716. Process for treating potable liquids.** F. S. BOARD and R. P. ROBICHAUX (assignors to Murray Deodorizers, Ltd.). U. S. Patent 2,516,099. 5 claims. July 25, 1950. *Official Gaz. U. S. Pat. Office*, **636**, 4: 1158. 1950.

Fluid dairy products are preheated to 110-160° F., then steam is injected to 162-185° F. while the fluid is passing through a vacuum zone ranging from 12-20 in. of Hg, then through a second vacuum zone ranging from 13-21 in. Hg for withdrawing vapors and gases. Following a 15-sec. holding period at 160-183° F. to effect pasteurization, the deodorized product is vacuum cooled.

R. Whitaker

**717. Freezing, hardening and dispensing cabinet and containers therefor.** L. A. M. PHELAN. U. S. Patent 2,517,234. 3 claims. Aug. 1, 1950. *Official Gaz. U. S. Pat. Office*, **637**, 1: 177. 1950.

A refrigerated cabinet provides refrigeration for a counter freezer mounted on top, a low-temperature hardening space and a dispensing compartment operating at dipping temperature.

R. Whitaker

**718. Agitating device for making frozen desserts.** W. B. MCGORUM. U. S. Patent 2,516,232. 13 claims. July 25, 1950. *Official Gaz. U. S. Pat. Office*, **636**, 4: 1191. 1950.

A motor-driven agitator for stirring mix, etc. in a tray in a household refrigerator freezing compartment is described.

R. Whitaker

**719. Apparatus for agitating and dispensing frozen foods.** T. CARVEL. U. S. Patent 2,491,852. 6 claims. Dec. 20, 1949. *Official Gaz. U. S. Pat. Office*, **629**, 3: 758. 1950.

Structural features are given for a freezer to manufacture soft ice cream and frozen custard. Mix is introduced into the rear of a horizontal cylindrical freezer where it is whipped with air, frozen and propelled toward the front of the freezer where it is withdrawn through a gate valve.

R. Whitaker

**720. Capping machine.** H. G. VORE (assignor to American Seal-Kap Corp.). U. S. Patent 2,516,278. 4 claims. July 25, 1950. *Official Gaz. U. S. Pat. Office*, **636**, 4: 1203. 1950.

Preformed skirted hood caps, impregnated with a thermoplastic adhesive, are heated as they pass through a high frequency electrostatic field, then placed on milk bottles and held in place until heat-sealed.

R. Whitaker

**721. Freezer.** L. S. MARANZ (assignor to Freeze King Corp.). U. S. Patent 2,515,722. 11 claims. July 18, 1950. *Official Gaz. U. S. Pat. Office*, **636**, 3: 934. 1950.



An arrangement on an ice cream or frozen custard freezer introduces mix from a supply tank into the freezing chamber. R. Whitaker

**722. Brine overflow attachment for ice cream freezers.** R. C. NIERSTE. U. S. Patent 2,514,787. 1 claim. July 11, 1950. Official Gaz. U. S. Pat. Office, 636, 2: 565. 1950.

To prevent brine from contaminating ice cream frozen in a verticle ice and salt freezer, an outer container is provided into which the brine overflows. R. Whitaker

**723. Emulsifying machine.** R. E. GODSBROUGH. U. S. Patent 2,514,992. 2 claims. July 11, 1950. Official Gaz. U. S. Pat. Office, 636, 2: 621. 1950.

A small capacity, 1-piston homogenizer has a built-in supply tank equipped with an agitator to premix the material and keep it uniform. A 2-stage homogenizing effect is obtained as the product is released through a series of slots after passage through a spring-loaded valve.

R. Whitaker

**724. Cooking apparatus.** N. J. PETERS (assignor to Damrow Bros. Co.). U. S. Patent 2,514,008. 1 claim. July 4, 1950. Official Gaz. U. S. Pat. Office, 636, 1: 279. 1950.

A horizontal screw-type cooker of the type suitable for making such products as process cheese has bearings so designed that the screw shaft may be removed from the trough for cleaning.

R. Whitaker

**725. Licking the ice and snow problem on loading platforms.** Anonymous. Natl. Butter & Cheese J., 41, 3: 38. Mar., 1950.

The use of iron pipe heating coils to melt snow and ice by placing them under the floor of the loading platform is illustrated. H. E. Calbert

**726. Maintenance of calcium chloride brine.** Anonymous. Milk Plant Monthly, 39, 6: 84-87. June, 1950.

Factors to be considered in the routine maintenance of refrigerating brines are proper strength, sufficient volume, freedom from ammonia, proper alkalinity and use of corrosion inhibitors. Detailed procedures for testing brine and tables for strengthening brine with  $\text{CaCl}_2$  are included in this article.

J. A. Meiser, Jr.

**727. Rust, billion dollar rat hole.** J. HOWARD. Am. Milk Rev., 11, 12: 26, 28, 43. Dec., 1949.

Rust and corrosion are a form of destruction of equipment, its cost annually in the world being estimated at upwards of 10 billion dollars. An estimated 2% of the entire tonnage of iron

and steel in service must be replaced each year because of loss through rust. Two corrosion control measures are described. One measure is to use corrosion-resistant alloys, such as stainless steel. The other is to use a protective coating. An improved deodorized and pre-oxidized fish oil is claimed to have considerable merit, particularly because it can be applied directly over a rusted surface. This oil will carry pigment, enabling its use as a final coat. D. J. Hankinson

**728. The problem of water scale formation.** W. F. BENSON, Limex Corp., Indianapolis. Natl. Butter & Cheese J., 41, 4: 34, 54. Apr., 1950.

The composition and amount of solids present in water that cause scale formation vary considerably throughout the country. The most common cause of scale formation is bicarbonate alkalinity that exists because of the amount of  $\text{CO}_2$  in solution. There is a balanced relation between soluble  $\text{Ca}(\text{HCO}_3)_2$ , gaseous  $\text{CO}_2$  and insoluble  $\text{CaCO}_3$ . The removal of  $\text{CO}_2$  by increased temperature, aeration or alkalinity promotes scale formation. These causes for the removal of  $\text{CO}_2$  cannot be prevented in ordinary operations. Therefore, water treatment is necessary to prevent the formation of scale on plant equipment. During the past 10 yr., several satisfactory water-treatment systems have been developed that are suitable for the average dairy plant.

H. E. Calbert

**729. How to pick the right valve.** J. MEYER, Minneapolis-Honeywell Regulator Co., Philadelphia, Pa. Am. Milk Rev., 12, 2: 34-37. Feb., 1950.

Valves either are hand-operated or automatic. Hand-operated valves are of the quick-opening or the proportional type. Proportional valves permit throttling control of the flowing medium. Automatic valves are diaphragm motor-operated (pneumatic), electric motor-operated or solenoid-operated. Many dairy plant operations are controlled by valve action. The valve engineer attempts to correlate price and performance in selecting the proper type of valve. In addition to proper design, high quality materials and satisfactory operation, the dairy company must be assured of repair service and replacement parts when needed.

D. J. Hankinson

Also see abs. no. 696.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**730. Milk and fat losses in processing fluid milk.** J. S. PFAUTZ, Penn. Milk Control Commission,

**Harrisburg, Pa. Milk Dealer, 39, 10: 45-47, 85-95. July, 1950.**

The butterfat and product pounds lost in the operation of a milk pasteurization plant are shown for the month of Sept., 1949. They are based on the total receipts in product pounds and butterfat pounds for 118 dealers handling 183,889,948 lb. of milk and milk products which represents from 65-68% of all the milk handled in the state of Pennsylvania. On the annual basis of these 118 dealers, the total cost of fat lost for all dealers, at the present support price of approximately 74¢/lb., would be approximately \$1,100,000 annually. Data is presented which summarizes the various products into standard milk. The routes are classified into retail and wholesale; returns of milk and cream were 4.63% for retail and 2.75% for wholesale routes. Data are given which show the loss in dumping of packaged milk and cream, loss in separating dumped milk and cream, the relation of the cost of dumped milk and cream to annual profit, receiving losses, filling losses, refrigerator losses, etc.

C. J. Babcock

**731. Inventory control for the milk plant. F. MERISH. Milk Plant Monthly, 39, 8: 32, 34-36. Aug., 1950.**

Fifteen sound reasons for the need of inventory control are discussed. Also included are certain forms for recording inventory data.

J. A. Meiser, Jr.

**732. What happens to the profits? M. J. KLUGER, Kapleau, Kluger and Co., Philadelphia, Pa. Am Milk Rev., 11, 12: 2, 3, 4, 6. Dec., 1949.**

This accountant's analysis of a firm's profit level makes use of another statement, the statement of sources and application of funds, in addition to the usual ones. This statement tells management if profits and other funds are properly used. Profits should not be confused with cash in the bank.

D. J. Hankinson

**733. The pallet system of material handling for the processing plants. L. H. HECKENDORN. Milk Plant Monthly, 39, 6: 28-30, 32, 34. June, 1950.**

As milk arrives in the cold box from conveyors, the cases are placed on pallets according to the driver's predetermined orders. At loading time, fork lift trucks transport the pallets to the delivery truck where the machine deposits its load. Empty bottle returns are stacked on pallets and removed by the mechanical lift to the conveyor feeding the bottle washer or stacked while awaiting washing. Cases may be tiered 12-15

case high on pallets without difficulty. Advantages of this system are that it: (a) reduces daily labor requirements, (b) speeds up loading and unloading of vehicles, (c) may be used for handling incoming supplies, (d) utilizes floor space more efficiently, (e) reduces length of conveyor lines, (f) reduces inventory losses and breakages caused by conveyor systems and (g) has flexibility.

J. A. Meiser, Jr.

**734. Dairy laundry reduces operating costs. J. LEE, Abbotts Dairies, Philadelphia, Pa. Milk Plant Monthly, 39, 8: 46-47. Aug., 1950.**

Dairy laundries reduce actual operating costs and eliminate waiting periods between pickup and delivery. Since about 90% of the work involves rough drying only, a limited amount of equipment is needed for starching and flatironing. Shipment of soiled and laundered clothes to and from the laundry is in locked drums. By working from 5:30 a.m. to 1:30 p.m., these drums containing laundered clothes can be returned the same day.

J. A. Meiser, Jr.

**735. Inter-dependency of dairy industry stressed. W. F. JONES. Can. Dairy Ice Cream J., 29, 6: 41-42. June, 1950.**

The more important factors which will have a decided influence on the long term position of the dairy industry are: (a) the extent to which substitutes are permitted to enter into competition with butter and other dairy products; (b) the level at which our national income and consumer purchasing power is maintained; (c) the prices at which dairy products are made available to consumers; (d) the availability and prevailing price levels in export markets for our surplus products; (e) the degree of government intervention in the marketing of our products; and (f) the extent to which we are prepared to promote the sale of our products in face of the ever-increasing competition for the consumer's food dollar.

H. Pyenson

**736. Abundant production wisely used. C. F. BRANNAN, Secty. of Agr. Am. Milk Rev., 12, 2: 5-6, 10, 65. Feb., 1950.**

Attention is called to the declining purchasing power of farm dairy products. Farmers deserve a fair return; therefore, a support program is needed. The purchase storage method of support is criticized because the consumer pays twice, once in the form of higher prices and again in the form of higher taxes. Instead, it is proposed that farmers be paid directly the difference between the average market price and the support level for the particular area. The support level would be based on the average purchasing power of cash

receipts from farm marketing during a recent 10-yr. period. This proposal has become known as the Brannan Plan. D. J. Hankinson

**737. History of dairy legislation key to dairy industry's progress.** H. F. GRIEG. *Can. Dairy Ice Cream J.*, 29, 7: 42-50. July, 1950.

Progress in dairy technology down through the years has been associated with 1 or a combination of 3 things: (a) the development of new tests, (b) the advance in dairy engineering and (c) the advent with each succeeding generation of new thinkers capable of grasping the advantages which the progress of the previous generation may have afforded. The author gives a summation of the dairy industry and the legislation which has played a part in the progress of milk products. H. Pyenson

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**738. Eliminating feed flavors during the grassy season.** F. FLAGG. *Milk Plant Monthly*, 39, 7: 34-35. July, 1950.

Mixing 1 can of grassy milk with 2,000 gal. of normal milk has been known to impart an objectionable flavor and odor to milk. This dairy attempted to eliminate this summer problem by mailing letters to producers in Feb. and March. Feb. correspondence explained how to control off-flavors in milk, and the March letters informed producers that no abnormal milk would be accepted. First offenders would be warned; second offenders would be suspended for 3 d.; and third offenders would be permanently excluded from shipping milk during the grassy season. This eliminated about 10% of the producers, although all but a few returned after control was gained. J. A. Meiser, Jr.

**739. Milking machine pulsator.** R. W. LEMM. U. S. Patent 2,517,327. 4 claims. Aug. 1, 1950. Official Gaz. U. S. Pat. Office, 637, 1: 201. 1950.

A device for producing pulsations in the vacuum supply of a milking machine is described. R. Whitaker

**740. Pulsator apparatus for milking machines.** J. R. LOWRY. U. S. Patent 2,516,328. 1 claim. July 25, 1950. Official Gaz. U. S. Pat. Office, 636, 4: 1216. 1950.

A self-contained milking machine on a small truck may be rolled from cow to cow. A motor-driven piston-type pulsator provides intermittent vacuum. The milk is collected directly in a milk can on the truck. R. Whitaker

**741. Pulsator apparatus for milking machines.** P. A. TAYLOR (assignor to Ideal Mfg. Co.). U. S. Patent 2,516,354. 1 claim. July 25, 1950. Official Gaz. U. S. Pat. Office, 636, 4: 1222. 1950.

Similar to abstract 740, but differing in mode of controlling pulsations. R. Whitaker

**742. Milk claw.** E. SHURTS. U. S. Patent 2,514,676. 3 claims. July 11, 1950. Official Gaz. U. S. Pat. Office, 636, 2: 537. 1950.

A manifold for milking machines with 4 outlets for attaching to teat cups is so designed that all interior surfaces may be inspected visually for sanitary condition. R. Whitaker

**743. Milk strainer.** D. O. BRANT. U. S. Patent 2,516,102. 2 claims. July 25, 1950. Official Gaz. U. S. Pat. Office, 636, 4: 1159. 1950.

A farmer's milk strainer in which the filter pad is held in place by a perforated rubber disk is described. R. Whitaker

**744. Dehorner.** W. I. COULL. U. S. Patent 2,516,959. Aug. 1, 1950. Official Gaz. U. S. Pat. Office, 637, 1: 106. 1950.

The horns of cattle are cut off by a blade which swings across a plate with a hole in it, the horn being inserted in the hole. R. Whitaker

**745. Mechanical barn cleaner.** L. E. PETERSON. U. S. Patent 2,516,798. 2 claims. July 25, 1950. Official Gaz. U. S. Pat. Office, 636, 4: 1335. 1950.

A device collects the material in a barn gutter and delivers it into a carrier or up on a pile located some point beyond the end of the gutter. R. Whitaker

**746. Cow tail holder.** A. J. ARENDS. U. S. Patent 2,516,744. 1 claim. July 25, 1950. Official Gaz. U. S. Pat. Office, 636, 4: 1320. 1950.

This device restrains a cow from swishing her tail. R. Whitaker

**747. Sucking device for calves.** P. O. STEVENS (assignor to Mutual Products Co.). U. S. Patent 2,516,730. 2 claims. July 25, 1950. Official Gaz. U. S. Pat. Office, 636, 4: 1317. 1950.

A vertical plate attached to a pail holds a tube which extends downward into liquid calf food in the pail. The upper end of the tube extends horizontally through the plate and terminates in a nipple. R. Whitaker

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**748. How, why and when ice cream gets into the home.** Anonymous. *Ice Cream Trade J.*, **46**, 7: 34-36, 38, 95, 96. July, 1950.

The American Dairy Association has just completed a market study based on interviews with the buying public and with manufacturer's retail outlets. The housewife buys 46% of the ice cream, while her husband purchases only 18%. Saturday is the most important day for ice cream purchases, while Friday and Saturday represent 50% of the "take home" purchases. Drug and food stores are the leading outlets and 4-8 p.m. are the hours of greatest purchases. Convenience determines why ice cream is bought at a certain outlet with brand preference a close second. Ice cream is being served in the home as a mealtime dish and backbar streamers rated the most effective advertising. W. H. Martin

**749. Sorbitol used in ice cream for diabetics.** P. H. TRACY and G. EDMAN, Univ. of Ill., Urbana. *Ice Cream Trade J.*, **46**, 7: 50-52, 83-84 July 1950.

Research aimed at improving the existing low-carbohydrate ice cream formula for this country's million diabetics has been conducted using hexahydric alcohol (sorbitol) as the source of sweetness. This compound is a member of the "sugar-alcohols" and is produced commercially by adding hydrogen to the aldehyde or ketone group of a sugar molecule. The value of sorbitol as a sweetener lies in the delay before it appears in the blood as glucose and its low carbohydrate and insulin demand values. Sixteen lb. of sorbitol solution and 5 g. of saccharin/100 lb. of mix produces ice cream of good body and sweetness comparable to a 14% sucrose ice cream. A good diabetic ice cream is produced using 17% fat, 5.1% s.n.f., 5% whole eggs, 16% sorbitol solution and 0.5% gelatin stabilizer. Overrun should be 70-80% to produce a good body. For the diabetic's convenience, the package should be labeled as to ingredients of the ice cream, explanation of the function of the ingredients and the total calories contained in the package.

W. H. Martin

**750. Fruit puree expected to play greater role in ice cream sales.** D. G. SORBER. *Can. Dairy Ice Cream J.*, **29**, 7: 37-39. July, 1950.

The production of frozen fruit purees is a recent development in the fruit processing industry. The purees now on the market are apricot, banana, boysenberry, lemon, orange, nectar-

ine, peach, plum, raspberry, strawberry and tangerine. Other purees can be made from apples, avocados, blackberries, loganberries, cantaloupes, cherries, cranberries, dates, feijoas, guavas, huckleberries, mangoes, passion fruit, persimmons and pineapple. The puree usually is added after defrosting at the freezer. A new frozen fruit dessert developed during the 2nd World War called Velve Fruit consists of fruit puree, sugar, gelatin and water. Sundae topping is another use for fruit purees. H. Pynson

**751. Lehigh Valley launches Duncan Hines ice cream.** Anonymous. *Ice Cream Trade J.*, **46**, 6: 30-32, 97. June, 1950.

The Lehigh Valley Cooperative Farmers of Allentown, Pa., have introduced a new deluxe pt. package of ice cream endorsed by Duncan Hines. Launched with promotional fanfare as an "Adventure in Good Eating", this ice cream contains 16% fat and low overrun with total solids slightly exceeding 42%. Within 2 wk., sales of the deluxe product equalled those of their standard pt. with no decrease in the sales of the standard pt. package. This quality product retails at 43¢ and the standard pt. at 26¢. W. H. Martin

**752. Measured portion, rectangular factory-filled package makes a debut.** Anonymous. *Ice Cream Trade J.*, **46**, 6: 52, 91, 92. June, 1950.

A new, versatile package for use either for consumption off the dealers' premises or for "measured portion" use at the fountain has been developed. The carton, in 3.2-6 oz. size, has a spoon affixed and is designed for eating directly from the package. This is particularly adaptable to vending machine use. In this new carton, without a spoon, a fountain operator has ice cream measured to go in a soda, sundae or banana split and has uniform service and a controlled portion cost. Reports from users listed advantages of speed, economy and cleanliness.

W. H. Martin

**753. Ice cream softener.** M. C. LUTERICK (assignor to Diced Cream Co. of America). U. S. Patent 2,516,895. 3 claims. Aug. 1, 1950 Official Gaz. U. S. Pat Office, **637**, 1: 90. 1950.

Ice cream is softened by agitation in this device which consists of a rotating disc and several times extending upward to form a basket-like agitator. The agitator fits into a container open at the top for loading and equipped with an outlet for drawing the softened ice cream. R. Whitaker

**754. A study of sherbets and ices** O. E. Ross, Natl. Pectin Prod. Co., Chicago, Ill., *Ice Cream Trade J.*, **46**, 7: 44, 45, 86-92. July, 1950.

As the sugar content is increased the body and texture is improved, flavor more distinct and acid flavor less sharp. Approximately 26% total sugar produces proper dipping qualities and good flavor, body and texture. When 27% of the cane sugar is replaced by corn syrup solids, better body and texture are produced. Addition of homogenized milk so that the sherbet contains 2% fat and 5.2% m.s.n.f. reduces the intensity of fruit flavor and tartness and produces a desirable sherbet. Frozen fresh orange and lemon juices are superior to either fresh juices or extracts or emulsions of citrus fruits in making a satisfactory sherbet.

W. H. Martin

#### 755. Newer methods in merchandising ice cream.

Anonymous. *Ice Cream Trade J.*, **46**, 7: 30-33, 99, 100. July, 1950

This Florida company has developed new methods in merchandising, plant procedure and distribution of packaged ice cream. A square pt. and 2 other packages, 1 containing 6 portions of vanilla, chocolate and strawberry and the other 20 portions, are manufactured. The ice cream is hardened, wrapped in corrugated board which then is wrapped and heat sealed in an attractive 4-color wax paper package in 1 complete, mechanical operation.

Attractive open display cabinets with colorful cards sealed in plastic were developed for use mainly in supermarkets to provide point-of-sale advertising for Velda-Plantation's trademarked name.

Insulated shipping containers of 50-gal. capacity on rollers were designed to provide easier handling for distribution. The latest equipment, including HTST pasteurization and a new automatic hardening machine, are housed in a beautiful, modern plant landscaped with tropical palms and flowers.

W. H. Martin

#### 756. Costing ice cream mix for the small dairy.

A. SEARLES, JR., Cornell's Dairy Prod., Endicott, N. Y. *Milk Plant Monthly*, **39**, 7: 40-42. July, 1950.

To itemize mix costs monthly profit and loss statements are made up. The first is a running inventory supply record of each ingredient in the mix excluding fat. The second is a mix manufacturing report on each batch of mix. The final statement is a summary of over-all costs including labor and overhead.

J. A. Meiser, Jr.

#### 757. Costs of ice cream distribution.

R. T. SMITH, Scranton, Pa. *Ice Cream Trade J.*, **46**, 6: 40, 41, 105. June, 1950.

The following are some of the trends ice cream distribution has been taking comparing 1949 with 1946 as based on a recent survey. Over 90% of the companies participating in the survey deliver to 50% of their dealers 2 d./wk. and 75% deliver to 30% of their dealers 1 d./wk.; the remainder are on an every-other-day 3, 4, 5, or 6 d./wk. delivery. Since 1946, 75% of the companies reported that their truck capacity has increased 21%. They are selling 47% more items, including flavors, and they deliver to 14% more dealers with the average cost 50% more in 1949, as compared to 1946.

W. H. Martin

Also see abs. no. 705, 717, 718, 719, 721, 722.

## MILK AND CREAM

### P. H. TRACY, SECTION EDITOR

#### 758. Fortified skim with solids added.

A. J. FERM, *Ferm Dairy*, Rockford, Ill. *Milk Dealer*, **39**, 10: 42, 54. July, 1950.

The *Ferm Dairy* of Rockford, Ill., is selling a product known as "Ferm's Vita-Skim." In preparing the product, 2.5% of skim solids and 0.75% butterfat are added to the regular skim. It then is fortified with 2,000 units of vitamin A and 400 units of vitamin D. The local health department cooperated by publishing several articles regarding low-fat milk plus solids and vitamins. Sales of the product increased from 3,000 qt. in Feb., 1950, to 7,000 qt. in Apr., 1950. The Vita-Skim sells for 16¢/qt., while the homogenized and creamline sell for 18.5¢/qt.

C. J. Babcock

#### 759. Supplementing fluid cream with frozen cream.

H. V. ATHERTON, *Can. Dairy Ice Cream J.*, **29**, 6: 62-64, 78. June, 1950.

Preliminary results indicate that frozen cream for use in a frozen cream-fresh cream mixture should be stored with 40% fat content rather than as 50% cream as is the storage practice for the ice cream industry. When 50% frozen cream is used for the mixture, a distinct skim layer is noted in the bottles. The frozen cream can be defrosted while the mixture is being heated to homogenizing temperature, slow defrosting of the cream producing no better results. 50% frozen and 50% fresh cream were used in this study. Best results were obtained using a homogenization temperature over 140°F. and a pressure of 100 lb./in.<sup>2</sup> on a single-stage machine. The trials, conducted only on a laboratory basis, produce a satisfactory coffee and whipping cream.

H. Pyenson

**760. Shall I homogenize?** R. F. HOLLAND, Cornell Univ., Ithaca, N. Y. *Am. Milk Rev.*, 12: 22, 24. Jan., 1950.

The decision to homogenize should be based on expected sales volume, influence of competition and profit margin. More problems are solved than solved by adopting homogenization. Some of the problems are: (a) high equipment cost, (b) additional costs for power, gaskets, oil, repair, maintenance, depreciation, cleaning materials and labor, (c) control of leucocyte type of sediment even when milk is clarified, (d) control of off-flavors such as sunshine, flat or chalky and rancid, (e) possibility of high bacteria counts, (f) seepage of milk past the bottle cap and (g) utilization of route returns.

D. J. Hankinson

**761. Carton carrier.** H. Z. GRAY. U. S. Patent 2,514,858. 7 claims. July 11, 1950. Official Gaz. U. S. Pat. Office, 636, 2: 585. 1950.

A hand carrier for holding several cartons of the Pure-pak paper milk bottle type is described.

R. Whitaker

**762. A bottle cap contest.** Anonymous. *Milk Plant Monthly*, 39, 7: 68-69. July, 1950.

To promote milk and dairy products to children, a bottle cap contest for boys and girls under 15 yr. of age was staged. Running for 2 mo., the contest awarded 278 prizes to the contestants, top awards going to the 3 persons having the largest number of bottle caps. 533 youngsters entered the contest and 976,764 caps were returned.

J. A. Meiser, Jr.

**763. Incentives promote sales.** F. FLAGG. *Milk Plant Monthly*, 39, 8: 38-40. Aug., 1950.

To promote the sale of homogenized vitamin D milk, routeman were given a 30-d. period to persuade their customers to change over from regular milk. For every customer converted and held an additional 30 d., a bonus of 40¢/qt. was given. Year round contests are held on a monthly gain basis. Using the previous month's points as a base, routemen receive \$1.00 for each point up to 10 and \$1.50 for each point above 10. Safe driving is promoted by the management setting up a kitty which is divided at the end of 3 mo. to those drivers not having an accident. These contests not only lift the morale of sales personnel, but they provide a strong incentive force as well.

J. A. Meiser, Jr.

**764. Routeman's contacts build sales.** Anonymous. *Milk Plant Monthly*, 39, 6: 56-58. June, 1950.

Routemen receive \$5.00 if they contact 30

prospects and \$10.00 for 50 contacts, regardless of whether they become customers. Records of these calls are maintained on file cards at the plant. Delivery men also receive an additional bonus of \$1.00 for every qt. of new business retained for 30 d.

J. A. Meiser, Jr.

**765. Postal cards bring new business.** Anonymous. *Milk Plant Monthly*, 39, 6: 68-69. June, 1950.

Penny postal cards distributed by routemen to customers request the name and address of friends, relatives or neighbors who may be interested in purchasing dairy products. These cards promote the sale of products, as well as providing the company with leads to prospective customers.

J. A. Meiser, Jr.

**766. New educational project for better understanding of the dairy industry.** Anonymous. *Milk Plant Monthly*, 39, 6: 60-61. June, 1950.

A new educational project known as a "Class Workit" has been prepared by Education Research, Inc, Washington, D. C., that will enable elementary students to assemble a 3-dimensional dairy plant in the classroom. With this model comes a supplement for teachers. Thus, the flow of milk from the farm through the plant processing equipment is fully explained. The project is not designed to replace plant visitation but to be used in conjunction with field trips.

J. A. Meiser, Jr.

**767. The cow—mankind's benefactress.** E. B. ANDERSON, United Dairies, Ltd., Central Lab., London. *Chem. Ind.*, 11, 195-204. Mar., 1950.

A discussion of the synthesis and nutritional value of the constituents of milk is followed by a summarizing review of several physical, chemical, microbiological and manufacturing aspects of butter, cheese, condensed milks and dry milk solids.

E. B. Collins

**768. The dairy industry in Greece.** S. A. KALOYEREAS, Agr. Expt. Sta., Louisiana State Univ., Baton Rouge. *Natl. Butter & Cheese J.*, 41, 3: 22-23, 49. Mar., 1950.

During the decade prior to the last war, the milk production in Greece was 555,000 tons. About half of the production was consumed fresh, the rest being made into butter and cheese. Butter production amounted to less than 5,000 tons annually. Though Greece exports some cheese (300 tons), its imports are more than twice its exports. Approximately 80% of the milk produced comes from sheep and goats. The per capita consumption of dairy products is low. An examina-

tion of the average Greek diet reveals it to be low in Ca and P. Annual per capita consumption of dairy products was as follows: 84 lb. of milk, 18 lb. of cheese, 1.57 lb. of butter. The milk industry is still in a primitive state of organization. Efficient milk producer cooperatives are needed. There also is a great need for modern equipment and technically trained personnel, as well as adequate sanitary regulations and composition standards. H. E. Calbert

**769. New Zealand dairy.** Anonymous. Milk Dealer, 39, 10: 43-44, 105-106. July, 1950.

The city of Wellington, N. Z., established the municipal milk department in 1918 and became the first city to have a municipally-owned milk plant. More than 140,000 consumers were supplied daily in 1948. In addition to these consumers, schools were supplied with milk under a government program which called for 279,670 gal. in 1948-49. Total sales of milk in this period were the highest ever recorded and amounted to 8,770,353 gal. Of this total gallonage, 6,607,032 gal. or 84.89% were bottled. The plant operation, marginal costs, farmer price system and consumer prices are discussed. C. J. Babcock

**770. F A O aiding European countries with equipment for milk production.** N. E. DODD. Can. Dairy Ice Cream J., 29, 6: 74-78. June, 1950.

The Food and Agriculture Organization of the United Nations is installing new equipment in many countries of the world where adequate dairy plants are lacking. H. Pyenson

Also see abs. no. 713, 720, 730, 731, 733, 738.

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**771. Factors affecting activity of chemical germicides.** C. K. JOHNS, Can. Dept. of Agr., Ottawa. Milk Plant Monthly, 39, 5: 54-56, 58. May, 1950.

Factors affecting the activity of chemical germicides are type of compound, concentration, time of exposure, temperature, organic matter, pH, wetting ability, stability of product, type of organism, condition of the organism, number of organisms present, nature and physical condition of surface, incompatible compounds and residual film. Maximum sanitizing effectiveness may be obtained if one applies this information to his operation. J. A. Meiser, Jr.

**772. Water and alkali required to wash milk bottles.** P. S. LUCAS, Mich. Agr. Expt. Sta., E.

Lansing. Am. Milk Rev., 11, 12: 44-45. Dec., 1949.

Studies on 1 bottle washer installation revealed that 0.86 gal. of soft water and 0.03 oz. of alkali were used to wash each qt. bottle or its equivalent. The water consumption rate should be useful in estimating water softening capacities for milk plants. D. J. Hankinson

**773. The fieldman and the farmer's wife.** GRACE R. DUFFEE and H. E. CALBERT, Univ. of Wis. Butter, Cheese & Milk Prod. J., 41, 6: 23-24, 42-43. June, 1950.

Surveys indicate that the farm wife does a large share of the milking and cleaning of milk utensils on the average dairy farm. A dairy plant fieldman must get the cooperation of the farm wife before any milk quality program that he is instituting can become effective. The home demonstration agent from the County Agricultural Extension Office, by her influence over the farm women, can assist the dairy fieldman in making a milk quality program a success.

H. E. Calbert

**774. A bug's eye view of dairy plant architecture.** E. M. SEARLS, Natl. Dairy Products Co., Inc. Butter, Cheese & Milk Prod. J., 41, 5: 32-33, 66-69. May, 1950.

An entomologist makes several suggestions to reduce the hazard of insect or rodent infestation when building or remodeling a dairy plant. Locate on side roads or rail tracks to avoid dust and dirt of heavy traffic. Rats and insects are more likely to be encountered when plants are located near lakes, rivers and centers of heavy population. Filtered air for ventilation, rather than air from open windows, reduces the chances of contamination by dirt, dust and insect fragments. Doors must be self-closing and be kept closed to be rodent proof. When false ceilings are used, they should be sealed off to keep out the dust and vermin. Some hiding places for such insects as roaches can be eliminated by setting electrical conduits and control boxes either flush with the wall and sealed or far enough away from the surface to permit easy cleaning. The fewer the conduits, pipes, etc. in the processing room, the less the number of hiding places for roaches and other insects. The storage of employees' lunches, cookies, etc. in lockers serves as an invitation to insects. Place lockers so that they can be cleaned on all sides and treated with insecticides.

H. E. Calbert

**775. Insects and rodents must be stopped before they start.** E. M. SEARLS, Natl. Dairy Prod.

Corp., N. Y. Am. Milk Rev., 12, 44-46. Jan., 1950.

The presence of insects or rodents is an index of sanitation. Effective control procedures should include (a) thorough clean-up of plant and out-

side areas, (b) effective screening and selfclosing devices for doors and tight passes for pipes and (c) use of effective residual type insecticides for insects and a systematic trapping program for rodents.

D. J. Hankinson

Also see abs. no. 695.





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## ABSTRACTS OF LITERATURE

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### BUTTER

O. F. HUNZIKER, SECTION EDITOR

**775. Moderne dansk smørfremstillingsteknik.** (Modern Danish butter manufacturing technique). H. HEDEMANN, Odense. Mælkeritidende, 63, 25: 543-547. June 23, 1950.

The various conditions under which Danish butter has been produced have contributed toward the standard which must be maintained. The conditions which the Danish butter experts consider important for producing their ideal butter are considered. Through the cooperation of Danish milk producers, creamery operators, Danish merchants and English buyers, a certain type of butter has been developed. This type of butter must have a clean, pleasant aroma, a smooth-textured, waxy consistency, a good spreadability, pleasing color and salt content satisfactory to the majority of customers. The moisture and fat contents must comply with the law. After 14 d. in storage at 13° C. the butter must not be leaky, show mold or have deteriorated. The aroma of Danish butter is considered to be its most outstanding characteristic; it results partly from products produced during ripening by bacteria and partly from the feed of the cows. Some of the older creamery operators have suggested that feed may have given a certain spicy aromatic quality to the butter, a flavor which they cannot seem to duplicate now when scarcely any weeds or wild flowers grow in grass and clover fields. Possibly, weeds and wild flowers can influence, in a minor degree, the tendency toward a slightly higher aroma development during cream ripening. Milk from cows allowed to graze along roadsides had a slightly better aroma production than milk from cows on good pasture lands which were free from most weeds and wild flowers. During the past 25 yr., there has been marked progress in equipment for handling milk and cream for butter-

making. It is unlikely that any roll-type churns are in use in Danish creameries today. The rollerless churn constructed of either stainless steel or wood has replaced the older types, but much still depends upon the man who operates the churn, regardless of how automatic it is. Reducing the tendency to oxidation in Danish butter still is a problem. In Sweden and Finland a special salt (A.I.V.) is used for the purpose of acid reduction, but this seems inadvisable for Danish butter. Any neutralizing agent, however sparingly used, might have an unfavorable influence upon the famed fresh aromatic flavor of Denmark's butter. When some butter appears to be more resistant to oxidation than some other it is not that the pH is too high but that the oxygen tension is lower than it ought to be.

The Danish butter industry has made marked progress in packaging butter in consumer-size packages of as fine quality as in the present wooden casks. The most efficient techniques for every phase of butter manufacture and marketing are being used to perpetuate the high reputation of Danish butter. G. H. Wilster

**777. Continuous butter working apparatus.** C. E. NORTH. U. S. Patent 2,521,398. 3 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, 638, 1: 217. 1950.

Freshly churned cream, with the butter in granular form is introduced into 1 end of this device, which consists of a horizontal cylinder, in which rotates a shaft holding 4 spiral-shaped blades adjacent to the cylinder wall. The butter is fed in at such a rate that it continuously falls from the rotating blades and moves forward because of the slight spiral shape of the blades. Wash water is sprayed on the top of the cylinder and withdrawn with the butter through a drain at the end of the cylinder. R. Whitaker

## CHEESE

A. C. DAHLBERG, SECTION EDITOR

**778. Ostens Gaeringsvarme (Fermentation temperature of cheese).** Anonymous. Danish State Experimental Creamery, Hillerød. Mælkeritidende, 63, 24: 524. June 16, 1950.

In order to measure the amount of heat produced in cheese during ripening, 2 identical wooden cabinets were built and placed in a room where the temperature was controlled automatically. Each cabinet was provided with a contact thermometer, a relay and a built-in lamp for supplying the necessary heat. The cheese was placed in 1 of these cabinets. The amount of heat produced in the cheese then could be determined by noting the difference between the amount of electricity consumed in the cabinet holding the cheese and in the empty cabinet. The cabinets were at 12° C., about 2° C. higher than in the room in which the cabinets were placed. A slight difference in the amount of electricity consumed in each cabinet, even when both were empty, made it necessary to measure the electricity used in each cabinet before and after the test, for periods of 3–5 d. Periods of 5–13 d. were allowed for the measurement of electricity consumed during the test. Since cheese containing mold had been shown to produce more heat than cheese without mold, Roquefort cheese was chosen for some of the experiments. The greatest amount of heat was produced during the period of greatest mold growth, about 8 d. after pricking.

Six cheeses with a combined weight of 20 kg. were used. The results showed a variation from 2.5–5.0 cal./kg. of cheese for each 24-hr. period. The marked variation in the heat could have been due to the method of measuring. Danish, Swiss and Gouda cheese, used for a similar experiment, were 5–6 wk. old when tested. The amount of heat produced in this cheese was not enough to be significant, since it was only about 0.5 cal./kg. of cheese for each 24-hr. period.

G. H. Wilster

**779. Process of making cheese.** C. TONE (assignor to Armour and Co.). U. S. Patent 2,520,183. 4 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, 637, 5: 1389. 1950.

Milled curd is filled directly into containers lined with an air-impervious wrapper and sealed. The cheese is kept in the container until cured.

R. Whitaker

CONDENSED AND DRIED MILKS;  
BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**780. Method and apparatus for gassing the contents of cans.** W. M. TOMKINS (assignor to Continental Can Co.). U. S. Patent 2,518,100. 12 claims. Aug. 8, 1950. Official Gaz. U. S. Pat. Office, 637, 2: 505. 1950.

Powdered or ground food materials such as milk, eggs, coffee, etc., packed in open-end cans, are gassed with nitrogen or other gas, prior to sealing the cans. A bell-shaped baffle is lowered into the center of the packed can until it is about 1 in. from the bottom. The gas is admitted into the bell and as it flows down through the powder and up on the outside it displaces the air. The bell is withdrawn and the can sealed.

R. Whitaker

**781. Method of heating food products in sealed containers.** I. A. V. E. CLIFFORN, G. T. PETERSON and J. M. BOYD (assignors to Continental Can Co.). U. S. Patent 2,517,542. 9 claims. Aug. 8, 1950. Official Gaz. U. S. Pat. Office, 637, 2: 364. 1950.

Liquid food products, such as evaporated milk, are sterilized rapidly by rotating the cans end over end about an axis located outside the cans and at such speed that the air bubble in the cans moves about half way up the sidewall of the can from the end and then returns to the end during 1 complete rotation of the can. This specific movement of the air bubble provides turbulence of the contents, resulting in rapid heating and cooling.

R. Whitaker

**782. Milk chocolate.** B. K. HALLQUIST and L. O. J. CAMPBELL (assignors to Svenska Mjolkprodukter Aktiebolag). U. S. Patent 2,519,833. 6 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, 637, 4: 1181. 1950.

Spray-dried milk powder having an average grain porosity of not over 10% by volume is used with cocoa, fat and sugar to make milk chocolate.

R. Whitaker

**783. Milk proteins and lactose from dried skim-milk.** S. R. HOOVER and E. L. KOKES, Eastern Regional Research Lab., Philadelphia 18, Pa. Ind. Eng. Chem., 42, 9: 1910–1912. 1950.

The recovery of protein and lactose from dried skim-milk by a countercurrent extraction process with water acidified with HCl was demonstrated. The dried skim-milk was leached in 4 stages with 5 times its weight of 0.25% NaCl at pH 4.1 to give a soluble extract of 14% lactose, whey salts

and added salt, riboflavin and minor constituents. The extracted solid consisted of the milk casein and heat-coagulable whey protein and contained 86% protein, 2% ash and 0-3% lactose. Lactose could be recovered by the usual processes of evaporation and crystallization. B. H. Webb

**784. Process of concentrating milk.** J. F. KOWALFWSKI and G. SPERTI (assignor to Institutum Div. Thomas Foundation). U. S. Patent 2,520,939. 5 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, **638**, 1: 99. 1950.

Pasteurized homogenized milk is concentrated by freezing to a slush and separating the ice from the concentrated milk solids. R. Whitaker

**785. Shortening.** G. C. NORTH, A. J. ALTON, and W. C. BROWN (assignors to Beatrice Creamery Co.). U. S. Patent 2,520,954. 1 claim. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, **638**, 1: 103. 1950.

A powdered shortening in which the fat particles are surrounded by a blend of non-fat milk solids and egg solids is described. R. Whitaker

**786. Casein manufacturing process.** P. F. SHARP (assignor to Golden State Co.). U. S. Patent 2,519,606. 5 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, **637**, 4: 1125. 1950.

Skimmilk is coagulated with a coagulant and at the same time an inert gas is injected in such a manner as to cause the coagulated casein to form a foam. The foam is floated off the whey and dried after washing with a water spray.

R. Whitaker

**787. Process for preparing casein.** J. A. REYMERS (assignor to Amino Acids, Inc.). U. S. Patent 2,518,493. 2 claims. Aug. 15, 1950. Official Gaz. U. S. Pat. Office, **637**, 3: 737. 1950.

A weak acid is added to skimmilk with continuous agitation at a temperature of between 2 and 16° C. The fine, flocculent pptd. casein is separated from the whey, washed, dried and used as a food supplement. R. Whitaker

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**788. Bactericidal efficiency of quaternary ammonium compounds.** C. T. BUTTERFIELD, E. WATTIE and C. W. CHAMBERS, Pub. Health Service, Cincinnati, O. Pub. Health Reports, **65**, 33: 1039-1056. Aug. 18, 1950

Bactericidal efficiency of 11 quaternary ammonium compounds used as active agents of 40

commercial sanitizers was determined by the method presented. Tests also were made for residual active agents. The nature of the water in which the compound was dissolved definitely influenced its germicidal efficiency. Interference induced by different waters occurred almost instantaneously and did not increase with time. This interference was reduced in some cases after removing dissolved gasses by boiling or aeration. Presence of even small amounts of soap or other detergents usually reduced action markedly. The higher the temperature of the solution used, within the usual range of 12-46° C., the more effective the toxic action. Changes in pH of the solution affect its activity but the direction of the change varied with the compound. A decrease in pH increased potency of some compounds and reduced that of others. Very unreliable results were obtained with test procedures available for measuring active bactericidal content of residuals. Because of this, it is essential to make bacterial examinations with the product under conditions in which it is to be used.

D. D. Deane

**789. Kan Syrningsvanskeligheder nu Effektivt Afhjaelpes? (Can starter failures be effectively prevented?)** A. J. ØVLBY. Maelkeritidende, **63**, 24: 526. June 16, 1950.

To some extent it has been possible to prevent starter failures due to bacteriophage by making a fog in the creamery rooms, using a 5% solution of hypochlorite disinfectant. However, since bacteriophage may be present in milk received daily at the creameries this method is only a partial control. To change from 1 source of starter culture to another has been of benefit. Two creameries which had experienced regular starter failures were able to prevent failure when a starter from the Utterslev creamery was used. Some bacteriophages were isolated by the dairy laboratory from a starter culture which had become inactive and these were found to possess specific characteristics. Some of the bacteriophages attacked the organisms in only 1 of the starters to which it was added. Some strains of bacteriophage were active against 2 of the starters, while others were active against 3 or 4 starter cultures. Commercial cultures, A, B, and C were affected by 6 of the 11 bacteriophages, while culture D was affected by 10 of these. The starter from the Utterslev creamery was not affected by any of the isolated bacteriophages. The starter from the Utterslev creamery had been used with good results for 21 yr. This starter was highly aromatic, with 20% of the isolated bacteria being beta-cocci. Of the streptococci present, 65% were good acid producers. This percentage was higher

than had commonly been found in starters and this might be the reason for this starter being particularly resistant to the action of the bacteriophage.  
G. H. Wilster

## DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

**790. Browning of ascorbic acid in pure solutions.** M. P. LAMDEN and R. S. HARRIS, M.I.T., Cambridge, Mass. Food Research, 15, 1: 70-89. Jan.-Feb., 1950.

Ascorbic acid heated in the presence of citric acid in pure solution underwent deepening of color. Other common organic acids cause a similar action. Increase in color and destruction of ascorbic acid did not depend on the presence of oxygen, but color was a function of the initial concentration of ascorbic acid. Furfural was obtained in the heating of ascorbic acid and citric acid at the boiling point but was not noted with dehydro ascorbic acid. These and other findings are discussed in their relationship to the browning reaction, especially as it pertains to citrus products.  
F. J. Doan

**791. Process for recovery of lactalbumin.** G. J. STREZYNSKI (assignor to the DeLaval Separator Co.). U. S. Patent 2,520,615. 19 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, 637, 5: 1500. 1950.

Whey, having a pH of between 4 and 7 is heated to a temp. of about 165-190° F. to ppt. the albumin. After cooling to not lower than 130° it is fed into a centrifuge from which the albumin is continuously discharged from the periphery and the whey and lactose from the inner part of the bowl.  
R. Whitaker

**792. Babcock test mixer.** G. F. MASSEY. U. S. Patent 2,520,556. 5 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, 637, 5: 1486. 1950.

A mechanical agitator mixes the contents of Babcock fat test bottles.  
R. Whitaker

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**793. Butterfat samples as affected by weigh can design.** V. SCHWARZKOPF, Lathrop Paulson Co., Chicago, Ill. Sou. Dairy Prod. J., 48, 1: 44, 46, 90-91. July, 1950.

Samples of milk taken further from the strainer in rectangular weigh cans when the milk is not properly agitated, are richer. Ten to 30 sec. are required for agitation by air or mechanically when

it is necessary. Sufficient mixing of the milk may be obtained from its own velocity alone in narrow weigh cans of medium length with not less than 1.5 in. pitch/ft. toward the outlet valve, with 3/16 in. perforated strainer set high and kept free from baffles or louvers, and having a minimum depth of about 8 in. at the front. The use of a blender to convert the small streams from the strainer into a heavy body of milk is recommended.  
F. W. Bennett

**794. Using an in-the-line milk filter as a sediment tester.** Anonymous. Milk Dealer, 39, 11: 42-43. Aug., 1950; Sou. Dairy Prod. J., 48, 3: 129-130. Sept., 1950.

A standard in-the-line milk filter equipped with sediment disc to take an accurate last minute sediment test on all milk handled in the plant is being used successfully at the Brook Hill Certified Milk Farms at Genesee Depot, Wis. The filter is made up of a series of stainless steel horizontal plates with cotton media, the suspension of which is controlled in the milk flow by stainless steel spiders. All milk must flow upward through this series of parallel pads which is placed between the dump tank and the cooler. Because of the upward flow of milk, the tops of the discs are clean, indicating that no foreign matter passes through to the finished product. The bottom, or upstream side of the pad, however, indicates whether any foreign matter has been introduced into the milk during either the milking or plant handling.  
C. J. Babcock

**795. Plastic coating protects dairy equipment surfaces against corrosion.** Anonymous. Milk Dealer, 39, 11: 51, 58. Aug., 1950.

Corrosite is a plastic which combines chemically with the metal surface it covers and, because it is non-porous, results in an anti-corrosive, acid-resistant surface that does not crack or peel. It hardens rather than deteriorates with age. Its use as a coating for pasteurizing, bottle-washing and bottle-filling equipment for milk at the Walker-Gordon Lab. Co., at Plainsboro, N. J., has demonstrated the practicability of plastics for the dairy industry in protecting metals exposed to daily washings, caustics, detergents and lactic acid. The successful use of vinyl film ("corrosite") on cement feed troughs on dairy farms also is reported.  
C. J. Babcock

**796. Size refrigerant lines for low cost.** Anonymous. Power, 94, 9: 91-93. Sept., 1950.

Cost of suction and discharge piping usually is a small part of the total outlay for a refrigeration plant, but undersizing piping can increase annual operating costs from 5-30% or more depending

on the pressure drop of the system. Piping of proper size will cause only a moderate pressure drop and will cost little more than undersized piping.

Excessive pressure drop in the suction line causes superheating of the suction vapor and causes the compressor to operate at a lower suction pressure. Low suction pressure causes poor operating economy and reduces the compressor capacity.

Undersized discharge lines from the compressor to the condenser causes a decrease in capacity and an increase in power input. Refrigerant pipe resistance depends upon the compressor capacity, vapor velocity, pipe length, number of bends and the pipe size. A table presents safe velocities for suction line and discharge line for ammonia, Freon-12, methyl chlorine and carbon dioxide.

H. L. Mitten, Jr.

**797. 19 ways operators ruin refrigeration equipment.** G. HOIMAN. Operating Eng., 3, 7: 36-37. July, 1950.

A list of the 19 most common mistakes is presented with a brief explanation and suggestion as to prevention.

H. L. Mitten, Jr.

**798. Hot tips on cold plants.** H. WELCH. Operating Eng., 3, 8: 36-37. Aug., 1950.

When welding flange joints and fittings, remove valve bonnet to vent line to atmosphere. Oil vapors in receivers and shell and tube condensers are dangerous when warm. Ammonia and oil vapors are explosive when mixed so that 17-27% of the total is ammonia.

Check valves should be installed on all compressor discharge lines directly above the unit. The angle-type is better than the horizontal because it operates noiselessly and cannot be jacked open. Pipelines should be free to expand and contract with temperature changes. They should also be accessible at all points for inspections.

Corroded bolts on flange joints should not be tightened while liquid lines are under full pressure. Corroded flange bolts may be removed from line under low pressure without shutting down, provided a C-clamp is placed on the flange and the bolts are cut and replaced one at a time.

Inspect and test controls and gauges regularly. The operator should be acquainted thoroughly with the local codes governing type of plant in his charge. Each member of the operating crew must be instructed in the use of safety equipment for emergencies.

H. L. Mitten, Jr.

**799. How to apply mineral wool heat insulation.** P. W. SWAIN, Power, 330 W. 42nd St., New York, 18. Power, 94, 9: 86-90. Sept., 1950.

Application of mineral wool insulation is presented pictorially with brief, to-the-point descriptions. The applications presented include between-masonry walls, between-metal sheets, wire-impaled blankets, types of supports, nail fastening, expansion joints, cement on brick wall, covers for tanks, and pipe and fitting covers.

H. L. Mitten, Jr.

**800. Grout—and here's how it's used.** J. J. O'CONNOR, Operating Engineer, Albany, N. Y. Operating Eng., 3, 7: 32-33. July, 1950.

Grouting has an advantage over shimming or wedging for machine bases because it takes up any unevenness in both concrete foundation and machine base so that the machine will rest firmly on the whole foundation rather than on a few points.

In setting a machine, pour the foundation and set the anchor bolts. See that foundation is clean and wet before machine is set in place and grout is poured. The best grout mix for most jobs is 1 volume cement to 2 volumes of sand. Water should be held to 6 gal./sack of cement. Shrinkage of grout increases and strength decreases as amount of mixing water increases. Before pouring, let grout set 2 hr. after it is mixed, then remix without adding water and pour immediately. This procedure will reduce settling. Use 1-1.5 in. of grout under bed plate edge. Pour through grout holes in base plate or under plate between base and forms. Special bases may require air venting.

H. L. Mitten, Jr.

**801. Being practical about oil viscosity.** Anonymous. Power, 94, 9: 117. Sept., 1950.

In sleeve bearings the journal load is supported by a continuous oil film. Maintenance of the film under varying speeds, loads and temperatures is the deciding factor in lubricant selection. With ball or roller bearings the oil film is not continuous because of the high unit pressure between the rolling elements and the races.

Most industrial designs provide large safety margins for oil viscosities so that machines can meet the practical variations in operating conditions. A chart presents recommended oil viscosities for various loads and operating speeds.

H. L. Mitten, Jr.

**802. Relief valve.** R. HINRICHS (assignor to Tri-Clover Machine Co.). U. S. Patent 2,521,166. 3 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, 638, 1: 157. 1950.

A spring-loaded pressure relief valve fitted into standard sanitary cross fitting is described.

R. Whitaker

**803. How good maintenance worked in a dairy.** G. GRIFFEL. *Operating Eng.*, 3, 8: 20-22. Aug., 1950.

Standby spares are kept for pumps, motors and drives. Spares for the main pieces of processing equipment cannot be justified because of cost.

The heart of the maintenance program is the report which provides for a request for maintenance by the production department and a report on parts, labor and comments by the maintenance department. These reports serve as guides for equipment replacements, budgets and maintenance scheduling.

Maintenance men can best be trained on the job. They should be permitted to go over each new piece of equipment with the manufacturer's representative. The shop should contain a supply of replacement parts, a lathe, drill press, power hacksaw, grinder, welding equipment and other portable tools. Special test rigs may be built and installed in the shop.

Chief engineer's job is to schedule maintenance, train men, requisition new equipment and sell value of maintenance to management.

H. L. Mitten, Jr.

Also see abs. no. 777, 781, 814, 815.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**804. Selection and inplant training of production men in the industry.** C. E. KREY, Sou. Dairies Inc., Washington, D. C. *Ice Cream Trade J.*, 46, 8: 56, 58. Aug., 1950.

A college student committee was set up to supervise training of a number of promising young men in college and those graduated. Each member is responsible for visiting certain allotted schools during the year and interviewing all dairy students interested in summer jobs or graduates seeking employment. Accepted students are placed on a 13-wk. summer schedule. A college graduate, new to the company, is put in a 60-wk. schedule designed to include every phase of the production work and prepare him as a supervisor of some department. With this system management is confident that alert capable and responsible men are supporting present production superintendents.

W. H. Martin

**805. Trends and influencing factors in consumption of dairy products.** L. SPENCER, Cornell Univ., Ithaca, N. Y. *Sou. Dairy Prod. J.*, 48, 3: 36-38, 40. Sept., 1950.

We now are eating more fruits and vegetables other than potatoes, more eggs, more dairy prod-

ucts, more fats and oils, much less bread and cereals and much less potatoes per capita than were consumed 40 yr. ago. In 1949 we consumed 9% more of all dairy products except butter than the average for 1935-39. There was a 38% decrease in the consumption of butter.

The increase in the consumption of oleo accounts for less than half the loss in the consumption of butter. The reduced consumption of bread and other foods on which to spread butter or oleo and the increased consumption of vegetables on which salad oil or dressings are used also are important factors in reducing butter consumption.

The quantities of dairy products the markets will absorb will be affected mostly by (a) trend and characteristics of population, (b) consumer incomes in relation to cost of living, (c) relative retail prices of foods, (d) developments in production and distribution and (e) consumer acceptance.

On Sept. 15, 1949, the average retail prices of dairy products had increased 85.3% and the average prices of all foods had increased 104.2% as compared with the 1935-39 average.

F. W. Bennett

**806. Examining ice cream distribution costs.** E. R. HUBBARD, Hubbard, Dilley and Hamilton, New York, N. Y. *Ice Cream Trade J.*, 46, 8: 34, 35, 86-88. Aug., 1950.

The magnitude of distribution costs, as well as their alarming increase—far in excess of the increased cost of labor involved—has prompted a study designed to increase efficiency of marketing and reduce distribution costs. A thorough knowledge of present distribution costs, obtained by an adequate accounting procedure, will evaluate costs relative to the various distribution functions performed. Wastes in internal distribution should be eliminated and distribution cost data from other manufacturers should be compared. When a logical system of distribution cost control is installed and operating, success is determined only by management studying facts it reveals and taking action where required.

W. H. Martin

## HERD MANAGEMENT

H. H. HERMAN, SECTION EDITOR

**807. Milking machine.** A. E. ANDERSON. U. S. Patent 2,518,589. 10 claims. Aug. 15, 1950. *Official Gaz. U. S. Pat. Office*, 637, 3: 761. 1950.

The novel feature of this milker is a manifold below the udder, so arranged that rearward counterpoise is provided when the front teat cups are

pulling and a forward counterpoise when the rear cups are pulling. R. Whitaker

**808. Overhead carriage and hoist for milk cans.** B. V. CULP. U. S. Patent 2,520,238. 2 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, 637, 5: 1403. 1950.

A motor-driven hoist mounted on an overhead rail facilitates placing cans of milk in a cooling tank, etc. R. Whitaker

**809. Gutter side wall cleaner for dairy barns.** C. A. GILBERT. U. S. Patent 2,519,645. 3 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, 637, 4: 1134. 1950.

The side walls of barn gutters are scraped clean by 2 beveled scraper blades and held against the side walls by a coil spring in a tube separating the 2 blades. A handle attached to the tube facilitates movement of the scraper along the gutter. R. Whitaker

**810. Animal confining means.** E. S. DIEHL. U. S. Patent 2,520,385. 7 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, 637, 5: 1440. 1950.

A stanchion for cows has a device for adjusting the size to fit the animal. R. Whitaker

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**811. Weight changes in packaged ice cream at cabinet temperatures.** J. A. MEISER, JR., Mich. State College, East Lansing. Sou. Dairy Prod. J., 48, 3: 26-27, 52, 54, 56. Sept., 1950.

Ice cream packaged in untreated pt. paper containers lost 16-29 g. during 12 wk. of storage at cabinet temperatures. Losses in weight in the individual packages were at a practically constant rate. Containers constructed of the heaviest paper and possessing the minimum surface area resisted desiccation of the ice cream to the greatest degree.

Containers coated with paraffin permitted losses in weight of only 0.3-6.3 g./pt. in 12 wk. Containers paraffined on both the inner and outer surfaces offered the greatest protection. Coating the containers with glassine or vinylite also retarded moisture loss. F. W. Bennett

**812. High serum solids content in quality ice cream.** A. J. GELPI, JR. and F. I. DOWDEN, La. State Univ., Baton Rouge. Sou. Dairy Prod. J., 47, 6: 42-44, 47-48. June, 1950.

In the experiment reported, mono- and diglycerides with high grade gelatin retarded crys-

tallization of lactose in mixes of high serum solids content, improved the whipping ability, produced a smoother and richer tasting finished product, decreased shrinkage and enabled the ice cream to withstand heat shock to a remarkable degree. The possibility of manufacturing a highly satisfactory ice cream containing 14.5-16% serum solids stabilized with 0.2% gelatin and 0.2-0.25% monostearate or other mono- or diglycerides was demonstrated. Ice cream from such mixes may be drawn at higher overrun and still meet legal requirements. F. W. Bennett

**813. Method of making ice cream layer cake.** G. A. ZABRISKIE and F. ZABRISKIE (assignors to Airline Foods Corp.). U. S. Patent 2,517,756. 7 claims. Aug. 8, 1950. Official Gaz. U. S. Pat. Office, 637, 2: 418. 1950.

Several thin rectangular wafers or crackers are held in notches on the sides of the carton, so as to make a series of compartments or spaces of equal size. Soft ice cream is filled into the spaces, the carton closed and placed at a low temperature to harden. R. Whitaker

**814. Mixing and scraping machine, especially adapted for use as ice cream freezer.** P. CARPIGIANI. U. S. Patent 2,519,543. 3 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, 637, 4: 1108. 1950.

A cylindrical container is caused to rotate by a shaft extending downward through the container from an overhead drive. A second rotating shaft between the drive shaft and container wall causes whipping and ice removal by a blade which is so formed that all inside surfaces of the container are scraped. R. Whitaker

**815. Apparatus for filling containers with ice cream, with cutter means and container controlled circuit breaking means for stopping the apparatus.** R. M. HESSERT. U. S. Patent 2,517,107. 4 claims. Aug. 1, 1950. Official Gaz. U. S. Pat. Office, 637, 1: 145. 1950.

This device, installed in an ice cream cabinet and driven by an exterior motor, mechanically packs pt. or qt. containers from bulk containers. The bulk container is inverted on a platform, a rotating blade cuts off small pieces which drop down into a screw conveyor which packs the pieces into the retail packages as they are sold. R. Whitaker

**816. Detachable cover and service bar for frozen foods containers.** W. S. FREDENHAGEN and M. S. SCHMIDT. U. S. Patent 2,518,134. 4 claims.



Aug. 8, 1950. Official Gaz. U. S. Pat. Office, 637, 2: 513. 1950.

This device is designed to convert a conventional ice cream cabinet into a display cabinet for self service stores or into a soda bar. It is placed on top of the cabinet after removing the sleeve covers. Wells for dispensing syrups, nuts, flavors, etc. provided, as well as sliding doors and a counter.

R. Whitaker

**817. Method of making ice cream sandwiches and to ice cream sandwiches and wrappers therefor.** L. D. OVERLAND. U. S. Patent 2,521,403. 11 claims. Sept. 5, 1950. Official Gaz. U. S. Pat. Office, 638, 1: 218. 1950.

Two edible wafers are held spaced and parallel by a paper wrapper. Ice cream from the freezer is filled into the space and the completed sandwich hardened.

R. Whitaker

**818. Precut ice cream cake and method of making same.** F. ADAMS. U. S. Patent 2,520,522. 11 claims. Aug. 29, 1950. Official Gaz. U. S. Pat. Office, 637, 5: 1477. 1950.

Pic-shaped pieces of ice cream are frosted on the sides and top and fitted together to form a complete circular unit.

R. Whitaker

**819. Ice cream dispensing package.** J. S. MILLER. U. S. Patent 2,519,271. 4 claims. Aug. 15, 1950. Official Gaz. U. S. Pat. Office, 637, 3: 942. 1950.

Ice cream is pushed out of the top of a cylindrical container by a second cylinder which telescopes into the bottom of the top container.

R. Whitaker

**820. Costing ice cream mix.** A. SEARLES, JR., Cornell's Dairy Prod., Endicott, N. Y. Ice Cream Trade J., 46, 8: 44, 45, 89 Aug., 1950.

After selling surplus milk on the market for 60-70% of cost, Cornell's Dairy decided to convert it to ice cream mix. The making of mix does not increase property tax, insurance, band cost or even depreciation of equipment, and general overhead is minimized if large scale production is not necessary. A form is completed for each batch made, listing types and amounts of ingredients used, labor costs and any overhead. Another form completed monthly, lists batches of mix made, value of mix, total labor charges and mix on hand; this acts as a check on the daily mix reports. The difference between gross sales and costs of production is only one profit; the hidden profit is the difference between mix sales, on a butterfat basis and the price the surplus butter-

fat would have brought if mix wasn't being manufactured.

W. H. Martin

**821. Gas station sites with ice cream stores.** Anonymous. Ice Cream Trade J., 46, 8: 30, 31, 95. Aug., 1950.

The Friendly Ice Cream Corp. has leased a retail outlet built and owned by the Atlantic Refining Co. An attractive colonial-type building is situated next to the gasoline stations. This arrangement by which gas stations and ice cream stores cooperate to the mutual benefit of both is expected to increase.

W. H. Martin

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**822. Effect of thyroxine on oxygen consumption and heart rate following bile duct ligation and partial hepatectomy.** B. GRAD and C. P. LEBLOND, McGill Univ., Montreal, Can. Am. J. Physiol., 162, 17-23. July, 1950.

In studies on male albino rats, these authors present confirmation of previous studies in which it has been maintained that the liver excretes and inactivates excess amounts of thyroid hormone in the body.

V. Hurst

**823. Influence of environmental temperatures and thyroid status on sexual development in male mouse.** M. MAQSOOD and E. P. REINEKE, Mich. State Coll., East Lansing. Am. J. Physiol., 162: 24-30. July, 1950.

Groups of young male mice were maintained at either 24 or 30° C. They were fed varying levels of thyroprotein or thiouracil and at the end of 3 or 4 wk. the animals were sacrificed and the testes and seminal vesicles weighed and sectioned.

Thiouracil fed alone depressed the weight of the testicles and seminal vesicles at both 24 and 30° C. Thyroprotein fed in dosages causing mild hyperthyroidism increased both testicular and seminal vesicle weight at 24 and 30° C. The optimal stimulating dosage of thyroprotein at 24° was 10 times the optimal stimulating dosage at 30°. Severe hyperthyroidism caused decreased testicular and seminal vesicle weights at both 24 and 30°. The dosage range of thyroprotein which increased testicular size at 30° was considerably more restricted than the range of dosages causing stimulation at 24°. Histologically, mild hyperthyroidism stimulated spermatogenesis and caused epithelial proliferation of the mucosa of the seminal vesicles, whereas hypothyroidism produced the opposite effects.

V. Hurst

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## ABSTRACTS OF LITERATURE

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### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**824 Medicator for cows' teats.** M. H. NEWELL. U. S. Patent 2,523,478. 8 claims. Sept. 26, 1950. Official Gaz. U. S. Pat. Office, **638**, 4: 1092. 1950.

A hand-operated device flushes cows' teats with liquid medication. R. Whitaker

**825. An important problem facing dairymen is ketosis and the dairy cow.** C. B. KNODE. Can. Dairy Ice Cream J., **29**, 9: 84. Sept., 1950.

See abs. no. 690, Oct., 1950.

**826. Brucella ring test antigen prepared by reduction of a tetrazolium salt.** R. M. WOOD, Johns Hopkins Univ., Baltimore, Md. Science, **112**: 86. 1950.

Details are given for carrying out the brucella ring test using a tetrazolium salt (4, 4'-bis) 3, 5-diphenyl-2-tetrazolinium)-biphenyl dichloride) instead of hematoxylin to stain the brucella antigen. The tetrazolium salt is reduced by living cells to an intensely colored violet-blue formazan. Apparently the reduction takes place inside the cell, and hence the antigenic specificity of the cell surface is not altered. Lots of antigen prepared over the last 2 yr. using the tetrazolium method all have been of uniform color intensity, specificity and sensitivity and have remained stable over prolonged periods under normal conditions of use and storage.

### CHEESE

A. C. DAHLBERG, SECTION EDITOR

**827. De controle op de kwaliteit van te exporteren kaas.** (Quality control for export cheese). F. KEESTRA, Zuivel-Kwaliteits controle-

Bureau-Z. K. B., Amsterdam, Holland. Neth. Milk and Dairy J., **4**, 2: 148-155. 1950.

The Z. K. B. (quality control bureau for dairy products) has handled in Holland the quality control for butter since 1937, dried milk since 1946, cheese since 1948. The Z. K. B. is an organization of the dairy industry under government supervision. Cheese may be exported only if the "Holland" brand of the Dutch cheese control is on it. This means that it has been checked for composition and purity. Neither can it pass the customs without an export certificate of the Z. K. B. for quality control. Quality requirements are of the negative type, several regulations mentioning what is not allowed. Thus, the cheese may not look bad from the outside or be out of shape. The rind may not have serious faults or cracks or have a wrong color. The inside may not have serious faults. Odor and taste may not be abnormal. A minimum age is required, 6 wk. in winter, 4 wk. during the summer and 2 wk. more if the cheese is sealed in paraffin or similar material.

There are no positive requirements because different countries and even parts of countries want different properties. The manufacturer places a brand on the cheese dealing with the composition and purity. The exporter has to place his number on every packing unit. From this the origin of the cheese always can be found out later on.

A. F. Tamsma

Also see abs. no. 837.

### CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**828. A use of ascorbic acid in frozen homogenized milk.** R. B. ANDERSON, C. W. BETZOLD and W. J. CARR, Sixth Army Area Food Lab.,

Seattle, Wash. Food Technol., 4, 7: 297-300. 1950.

Milk was processed after ascorbic acid had been added at the rate of 0, 1.5, 3.0, 6.0 and 12.0 g./100 lb. of milk. The milk was pasteurized at 75° C. for 15.8 sec., homogenized at 58-60° C. under 1700 lb. pressure, cooled to 3° C., placed in commercial qt. paper cartons and stored at -17.8 to -16.7° C. for 30, 60 or 90 d. At the end of the storage period the samples were thawed 8 hr. at 19-20° C. then held 15-17 hr. at 4.4° C. Samples fortified with 0, 1.5 and 3 g. of ascorbic acid/100 lb. of milk had a strong, definite or slight oxidized flavor, while the samples fortified with 6 or 12 g. of ascorbic acid/100 lb. of milk were free from off-flavors. Approximately 1.25 g. added ascorbic acid were expended in protecting the flavor of the milk during processing and 30-d. storage. Milk fortified to the 6-g. level contained approximately 128 mg. of vitamin C./qt. after 30 d. of storage and 108 mg./qt. after 90 d. storage.

E. R. Garrison

**829. Meringues and method of making the same.** J. A. SNELLING (assignor to Proctor and Gamble Co.). U. S. Patent 2,524,333. 12 claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, 639, 1: 153. 1950.

Nonfat dry milk solids are mixed with not over 9% by weight with a mixture of alkali and alkaline earth sulphates and chlorides and an edible acid in such proportions that when 3-10 parts by weight of water is added to each part of milk powder in the meringue powder, the pH will be between 5 and 7.

R. Whitaker

**830. Process for the production of artificial bristles and the like from protein.** T. L. McMEEKIN, T. S. RIED, R. C. WARNER and R. W. JACKSON (assignors to U. S. A., as represented by Secy. of Agr.). U. S. Patent 2,521,738. 5 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, 638, 2: 415. 1950.

A fibre having a tensile strength of not less than 0.8 g./denier, is made by kneading iso-electric casein with water at 80-100° C. until plastic, extruding into air at 95-110° C., stretching the filament, treating with an anti-sticking agent, followed by hardening in a bath, stretching again and rehardening before drying under tension.

R. Whitaker

**831. Recovery of lactalbumin.** G. JOSH and M. E. HULL (assignors to Armour and Co.). U. S. Patent 2,521,853. 6 claims. Sept. 12,

1950. Official Gaz. U. S. Pat. Office, 638, 2: 444. 1950.

A coagulable protein is added to whey and the pH adjusted to 4-5. The mixture then is heated and the liquid drained off the 2 coagulated proteins.

R. Whitaker

**832. Process for the manufacture of foam producing albuminous products and their application in foodstuffs and luxuries.** J. LENDERINK. U. S. Patent 2,522,050. 10 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, 638, 2: 494. 1950.

Casein or other protein is hydrolyzed at about pH 10 with  $\text{Ca}(\text{OH})_2$  and  $\text{Mg}(\text{OH})_2$  at a temperature below boiling for at least 2 d. until the mixture contains 5-40% polypeptides and has strong foaming properties.

R. Whitaker

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**833. Isolements de ferments lactiques particuliers au lait de brebis et au fromage de roquefort. (Isolation of lactic acid fermentors characteristic of sheep's milk and roquefort cheese.)** C. ALAIS. Lait, 30, 297: 349-359. July-Aug., 1950.

Lactic acid cultures isolated from roquefort cheese and from sheep's milk exhibited distinctly different characteristics when cultured in sheep's milk as compared with cow's milk. Strength of the cultures when carried in sheep's milk remained high for protracted periods. In cow's milk, the organisms rapidly lost capacities to produce acid and to inhibit contaminants. These cultures, carried in sheep's milk, were observed to yield excellent results when used in the manufacture of roquefort cheese; however, they were entirely unsatisfactory when employed in production of blue cheese (made from cow's milk). Reasons for preferential growth of cultures in sheep's milk are discussed.

S. Patton

**834. Recherche, dans le lait en nature de certaines bacteries pathogenes pour l'homme. (Examination of raw milk for certain bacteria pathogenic to man.)** G. GUILLLOT, A. NEVOT and G. THEULIN. Lait, 30, 297: 337-349. July-Aug., 1950.

Methods for detecting the principal bacteria, pathogenic to man and incident to milk, are presented and discussed.

S. Patton

**835. The effect of hypochlorite and quaternary ammonium compounds, used in udder washes, on the chemical composition and bacterial flora on the milk produced.** E. M. KESLER, C. B.

KNODT and J. J. REID, Penn. State College. *J. Milk & Food Technol.*, **13**: 288-291. Sept.-Oct., 1950.

One quaternary ammonium compound (200 ppm.) and 200 and 400 ppm. concentrations of chlorine were compared with clean water in this study. Both sanitizers were considered equally ineffective when used under comparable conditions in checking the spread of organisms usually associated with mastitis. Although a general reduction of the udder microflora of the cows was noted, no apparent differences were observed between treatments on the chloride content or pH values of the milk produced.

II. H. Weiser

**836. Antibiotics in milk and discussion of problems encountered.** F. J. DOAN. *Can. Dairy Ice Cream J.*, **29**, 9: 35-36. Sept., 1950.

Antibiotics such as penicillin, aureomycin, sulphamethazine and streptomycin, used for the treatment of mastitis infections in the udders of producing dairy cows, have been reported in the milk from such cows for several milkings after treatment. In many cases arrested acid development has resulted when such milk is used in the manufacture of various types of cheese and buttermilk. At present, the only satisfactory control of the problem of antibiotics in milk is to try to get the producer to keep the milk from treated udders out of the milk shipped to the dairy. It probably is best to discard no less than 3 milkings following the treatment.

H. Pyenson

**837. Preliminary report of effect of mastitis curatives on cheese making.** A. BRADFIELD. *Can. Dairy Ice Cream J.*, **29**, 9: 37-38. Sept., 1950.

The results, so far, indicate that problems may be expected in cheese making if the newer methods of mastitis treatment which depend upon the use of antibiotics become common.

H. Pyenson

**838. The site of action of penicillin. 1. Uptake of penicillin on bacteria.** D. ROWLEY, P. D. COOPER and P. W. ROBERTS, St. Mary's Hospital, Paddington, and E. L. SMITH, Glaxo Laboratories, Ltd., Greenford, Middlesex. *Biochem. J.*, **46**, 2: 157-161. 1950.

The preparation of radioactive penicillin, using  $^{35}\text{S}$  in the medium, is described. By tracing this radioactive penicillin, the amount of penicillin attached to the bacterial cells can be estimated. The action seems to be due to a direct chemical reaction. The penicillin concentration attained inside "sensitive" or growing bacterial cells was much greater than in the medium, but for resistant

or resting cells it was much less. Attempts were made to block the uptake of penicillin, as well as to remove the attached penicillin from bacterial cells.

A. O. Call

**839. The microbiological determination of pyrimidines with lactobacilli.** R. B. MERRIFIELD and M. S. DUNN, Univ. of Cal., Los Angeles. *J. Biol. Chem.*, **186**, 1: 331-341. Sept., 1950.

An assay procedure for free and combined pyrimidines has been developed employing *Lactobacillus brevis* (ATCC 8287) and *L. helveticus* (ATCC 335). Only uracil and thymine were found active toward *L. helveticus*, which revealed a strict requirement for free pyrimidines. *L. brevis*, however, utilized both free and combined pyrimidines and exhibited essentially the same activity toward uracil, cytosine, orotic acid, uridine, cytidine, diammonium uridylate and cytidylic acid. Pyrimidine concentrations employed during assay were 0.3-5.0  $\gamma$  uracil/ml. medium with *L. brevis*, 0.3-1.0  $\gamma$  uracil/ml. medium with *L. helveticus* and 0.27-1.07  $\gamma$  thymine/ml. medium with *L. helveticus*.

H. J. Peppler

## DAIRY CHEMISTRY

II. H. SOMMER, SECTION EDITOR

**840. Alanine, glycine and proline contents of casein and its components.** W. G. GORDON, W. F. SEMMETT and M. BENDER. *E. Reg. Research Lab., Philadelphia, Pa.* *J. Am. Chem. Soc.*, **72**, 9: 4282. Sept., 1950.

By means of the radioisotope derivative technique whole casein and its 3 components,  $\alpha$ -,  $\beta$ - and  $\gamma$ -casein, were analyzed for alanine, proline and glycine. Results corrected for moisture and true ash reveal that whole casein contains 3.2% alanine, 2.0% glycine and 10.6% proline. These values are in close agreement with those reported in the literature.

H. J. Peppler

**841. Rancidity in milk and cream and discussion of milk-lipase.** E. G. HOOD. *Can. Dairy Ice Cream J.*, **29**, 8: 58-62. Aug., 1950.

The defect produced by milk-lipase action commonly is called rancidity. Conditions must be suitable for lipase activity, otherwise it cannot break down the fat.

The enzyme usually shows the most activity at a temperature of 37-40° F. and a pH of 8.4-8.6. Traces of heavy metals have an inhibiting action on milk lipase. Rancidity is most likely to occur when the cows are in advanced stage of lactation and have been milking for a year or more without freshening. Cows at the

end of lactation period also may produce rancid milk. Green feed will reduce the incidence of rancid flavors in milk. Rancidity can be induced by homogenization at a temperature under 130° F., violent agitation and by a temperature treatment—precool to 40° F., reheat to 80° F. and recool to 50° F. Milk produced in winter months is more subject to rancidity than milk produced in late spring and summer.

H. Pyenson

**842. Le colostrum et le lait dans leur rapports avec l'immunité du jeune. (Colostrum and milk in connection with immunity of the young.)** E. LEMETAYER, L. NICOL, O. GIRARD, R. CORVAZIER et M. CHEYROUD. *Lait*, **30**, 297: 359-373. July-Aug., 1950.

The work concerning placental vs. colostrum transmission of immunity to the newborn of a number of mammals is reviewed at length. The study deals with levels of antibodies in the blood and colostrum of mares vaccinated or hyperimmunized against tetanus or diphtheria. In the case of the mare, the prepartal colostrum invariably carries a higher level of antibodies than does the blood. The colostrum level drops very rapidly at the time of birth. The authors propose that hormone-induced changes in the gland and dilution effect, due to increase in the quantity of secretion are responsible for reduced antibody titre in colostrum at birth.

S. Patton

## DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

**843. A study on the performance of a side-opening milk cooler.** G. H. WATROUS, JR. *Can. Dairy Ice Cream J.*, **29**, 9: 44-46. Sept., 1950.

No significant differences were noted in bacterial levels obtained on milk cooled in the side-opening spray-type cooler, as compared to the conventional immersion-type cooler, either with or without water agitation in the latter. The side-opening cooler cooled milk below 50° F. in less than 45 min. compared to 8.5 hr. without agitation and 2.25 hr. with agitation in the immersion-type cooler. The final temperature of the milk in the side-opening cooler varied between 42° and 45° F. In the immersion type cooler the final temperature ranged between 34.4 and 39.5° F. No significant difference in electricity consumption with either cooler was noted.

H. Pyenson

**844. Apparatus for the production of ice cream.** D. WESTMORELAND. U. S. Patent 2,524,616. 5

claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, **639**, 1: 224. 1950.

A continuous ice cream freezer consisting of 3 horizontal cylinders one above the other is described. Mix enters the top cylinder, where air is incorporated by a rotating hollow dasher and some cooling takes place. From the top cylinder the ice cream flows to the middle and then to the lowest cylinder, both of which are equipped with rotating blades which scrape the cylinder walls. The jacket of the bottom cylinder is flooded with boiling refrigerant, which expands to a gas in the jacket of the middle cylinder and then flows through the jacket and hollow dasher of the top cylinder.

R. Whitaker

**845. Automatic control for the freezing of ice cream.** A. J. TACCHELLA (assignor to Steady Flow Freezer Co.). U. S. Patent 2,522,648. 17 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638**, 3: 773. 1950.

As ice cream is withdrawn for serving from this freezer, additional mix and air are automatically admitted to maintain a constant overrun.

R. Whitaker

**846. Frozen custard machine.** B. H. WOODRUFF. U. S. Patent 2,523,853. 16 claims. Sept. 26, 1950. Official Gaz. U. S. Pat. Office, **638**, 4: 1191. 1950.

A freezer for making soft ice cream, frozen custard, etc. consists of a horizontal refrigerated cylinder with a rotating dasher and scraper blades. Mix is metered from a supply tank into an inlet in the freezer in proportion to the amount of soft frozen product withdrawn for serving.

R. Whitaker

**847. Scraper for freezing apparatus.** C. ERICKSON and E. SPELLMAN. U. S. Patent Reissue 23,267. 15 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638**, 2: 397. 1950.

A scraper blade, pivoted on arms attached to the dasher of an ice cream freezer, is so designed that it may be easily removed for cleaning.

R. Whitaker

**848. Pasteurizing system.** R. E. OLSON and G. E. HELLER (assignors to Taylor Instrument Co.). U. S. Patent 2,522,796. 7 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638**, 3: 810. 1950.

An electrical system of controlling the temp. in a high-temp., short-time milk pasteurizing system, including milk-to-milk regeneration, a final milk to water heater and a flow diversion valve is described. The flow diversion valve is actuated

by either a decrease in temp below that desired or by an increase in the desired velocity of the milk flow.  
R. Whitaker

**849. Can washer.** A. W. SMITH (assignor to Rice and Adams Corp.). U. S. Patent 2,522,310. 11 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638**, 2: 561. 1950.

A straight-line milk can washer of the rising jet type is described.  
R. Whitaker

**850. Label holder for milk cans.** S. PETERSEN. U. S. Patent 2,522,398. 1 claim. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638**, 2: 585. 1950.

A slide is provided on a cross arm of milk can lids for holding a removable label for identifying the can and contents.  
R. Whitaker

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**851. The utilization of non-protein nitrogen in the bovine rumen. 5. The isolation and nutritive value of a preparation of dried rumen bacteria.** M. L. McNAUGHT and J. A. B. SMITH, Hannah Dairy Research Inst., Kirkhill, Ayr, and K. M. HENRY and S. K. KON, Univ. of Reading. Biochem. J., **46**, 1: 32-36. 1950.

Batches consisting of 2-3 l. of bovine rumen liquid were taken from a fistula. They were incubated with added maltose and urea and the rumen bacteria then separated by a Sharples super-centrifuge. This procedure was repeated until a total of 130 l. were processed. The yield was about 3.5 g. bacteria/l. The conversion of non-protein N to protein is demonstrated. In composition the dried rumen bacteria are similar to dried yeast. In biological value the material compares favorably with "dried-milk protein."

A. O. Call

**852. The utilization of non-protein nitrogen in the bovine rumen. 6. The effect of metals on the activity of the rumen bacteria.** M. L. McNAUGHT, E. C. OWEN and J. A. B. SMITH, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **46**, 1: 36-43. 1950.

The effects of various concentrations of Cu, Co, Mo and Fe on the development of rumen bacteria in rumen liquid were studied using *in vitro* techniques. The tolerated and toxic levels (in ppm.) were Fe, 100 and 1000; Cu, 10 and 25; Co, < 10 and 1000; Mo, 100 to 1000 and 2000. The effects of several organic chelating agents also were studied.  
A. O. Call

**853. Deposit and residue of recent insecticides resulting from various control practices in Cali-**

**fornia.** W. M. HOSKINS, Univ. of Cal., Berkeley. J. Econ. Entomol., **42**, 6: 966-973. Dec., 1949.

DDT was used on alfalfa for insect control. The hay was fed to dairy cows. DDT in the milk measured 15-23% of the DDT intake on the feed. Benzene hexachloride appeared in cow's milk within 24 hr. after being sprayed on the cow. Other data of DDT, DDD and parathion on alfalfa are included.  
E. H. Fisher

Also see abs. no. 842, 857, 858.

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**854. Milking machine pulsator.** S. P. WALL (assignor to Rite-Way Prod. Co.). U. S. Patent 2,523,795. 9 claims. Sept. 26, 1950. Official Gaz. U. S. Pat. Office, **638**, 4: 1175. 1950.

A device for causing pulsations in the vacuum line of a milking machine is described.

R. Whitaker

**855. Teat cup claw.** W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,524,193. 5 claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, **639**, 1: 117. 1950.

A four-outlet manifold for connecting the teat cup tubes of a milker to an intermittent vacuum supply is described.

R. Whitaker

**856. Milker timer.** W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,524,194. 5 claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, **639**, 1: 118. 1950.

A device causing pulsations in a vacuum supply for milking machines is described. R. Whitaker

**857. Calf feeder.** F. J. HABERKORN. U. S. Patent 2,522,820. 3 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638**, 3: 816. 1950.

A calf feeder consisting of a lid containing an outlet terminating in a nipple, which fits on the top of a cylindrical vessel holding liquid calf food is described. The device is placed in operation by inserting it in a holder attached to a wall, which holds it so the nipple is on the bottom.

R. Whitaker

**858. Calf feeder.** H. J. LARSON. U. S. Patent 2,522,757. 8 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638**, 3: 801. 1950.

A tube, mounted in a vertical wall, extends to the bottom of a pail of liquid calf food, the upper end terminating in a nipple.  
R. Whitaker

**859. Weaning basket.** L. E. COX. U. S. Patent 2,523,820. 1 claim. Sept. 26, 1950.

Official Gaz. U. S. Pat. Office, 638, 4: 1182. 1950.

A basket-shaped device for covering a cow's udder is held in place by straps over the cow's rump and back. R. Whitaker

**860. Device for assisting parturition of animals.** B. N. FRANK. U. S. Patent 2,522,508. 4 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, 638, 3: 738. 1950.

An obstretical device for assisting calving in cattle is described. R. Whitaker

**861. Handcart for milk cans and the like.** R. E. PUTMAN. U. S. Patent 2,522,894. 4 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, 638, 3: 834. 1950.

A 2-wheeled cart for easily transporting a can of milk is described. R. Whitaker

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**862. Chocolate ice cream and discussion of formula.** C. W. DECKER. Can. Dairy Ice Cream J., 29, 8: 78-82. Aug., 1950.

Chocolate ice cream represents approximately 15% of the total ice cream sales. Dutch process cocoa containing 20-22% cocoa fat produces a chocolate ice cream without bitterness or harshness. 1.5% chocolate liquor or blend, 3% coca and 18% sugar makes a good chocolate ice cream. A portion of the liquor may be replaced by cocoa at the rate of 0.25% cocoa for each 0.5% chocolate liquor. Any changes in the chocolate ice cream formula should be made gradually and preferably in the slack season of the year. H. Pyenson

Also see abs. no. 844, 845, 846, 847.

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**863. Packaging whipping cream in pressurized containers.** E. GRAHAM. Crown Can Co., Philadelphia, Pa. Food Technol., 4, 6: 225-229. 1950.

The development of single-trip metal containers for pressurized whipped cream and the principle of whipping by effervescence are outlined. To properly pasteurized cream containing approximately 30% butterfat are added 5-10% of sugar, vanilla flavoring and stabilizer (dehydrated egg albumen, sodium caseinate, gelatin, skim milk powder). Usually 7 fluid oz. of the mix are placed in the 12-oz. pressure container on regular dairy fillers, the metal gasketed cap with valve

assembled is clinched to the top, then the cans pass to the gasser. The gas ( $N_2O$  or 85%  $N_2O$  and 15%  $CO_2$ ) is added through the container valve to yield an equilibrium pressure of 75-90 p.s.i.g. The gassed container then is shaken vigorously for 10-30 sec. to hasten equilibrium between gas and mix and to partially clump the butterfat. An average overrun of about 250% is obtained or a yield of 25 fluid oz. of whipped cream from the original 7 fluid oz. Since the internal pressure tends to drop as the contents are dispensed from the container, the overrun of the whipped cream decreases accordingly. A "dry" firm whip is desired. A "wet" whip is associated with a low equilibrium pressure and insufficient agitation. Drainage varies inversely with the butterfat content and the gas pressure but can be decreased by the addition of stabilizer. Homogenized cream whips equally as well as regular cream by the aeration process but requires more agitation after gassing and therefore, generally is not used. Homogenized cream, however, shows less tendency to creaming and plugging and requires less shaking by the housewife before using.  $N_2O$  is regarded as being non-toxic and is widely used as an anesthetic. The gas (85%  $N_2O$  and 15%  $CO_2$ ) exerts a bacteriostatic effect upon the cream but the product should be stored in a refrigerator until dispensed. E. R. Garrison

**864. Cream separator.** B. F. DOSCHER. U. S. Patent 2,523,561. 4 claims. Sept. 26, 1950. Official Gaz. U. S. Patent Office, 638, 4: 1114, 1950.

A device for removing the cream from the top of a cream-top type of glass milk bottle, without mixing with the skim layer is described.

R. Whitaker

**865. Bottle crate.** D. T. TICHENOR (assignor to United Steel and Wire Co.). U. S. Patent 2,519,800. 4 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, 637, 4: 1173. 1950.

A wire milk bottle crate is described.

R. Whitaker

Also see abs. no. 848.

## NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

**866. Production of milk substitutes.** L. NICHOLLS. Food Manufacture, 25, 3: 95. 1950.

This reviews the potential application of nutritional substitutes (particularly in areas such as topics where milk generally is unavailable), the protein and vitamin dietary requirements and the use of soya "milk". K. G. Weckel

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**867. Quaternary ammonium compounds as sanitizers and cleaner sanitizers.** P. R. ELLIKER. *Can. Dairy Ice Cream J.*, 29, 8: 64-66, 76, 84. Aug., 1950.

Some quaternary ammonium compounds are combined with non-ionic wetting agents and certain alkaline cleaning compounds to provide a combination cleaner or detergent and sanitizer. Quaternary ammonium compounds sometimes termed cationic, surface active agents, form a deposit on the surface of equipment, producing a germicidal or bacteriostatic film. They are characterized by a high degree of stability. Whether these compounds are toxic to humans has not been settled. In general, quaternaries are effec-

tive in destruction of Gram-positive bacteria but usually are slower than hypochlorites in destruction of Gram-negative bacteria. Quaternaries appear to be less effective in destruction of bacterial spores than are the hypochlorites but seem to be able to prevent germination of spores and growth of spore-forming types. As little as 10 ppm. quaternary in milk may seriously retard growth of lactic acid starter bacteria in starters, cultured milk or cheese milk. Organic matter definitely interferes with germicidal activity of quaternary ammonium compounds. Hard water salts, such as those containing Ca, Mg and Fe tend to inactivate quaternaries. The eosin titration method may be used to determine concentration of quaternary ammonium compounds.

H. Pyenson

Also see abs. no. 834.





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